

## Answers and Questions from Paris

THE meeting of the heads of government of the enlarged European Community in Paris last week is at once a challenge to the sceptics, on the mainland and elsewhere, and a sign that even the enlarged community is in danger of making progress by setting somewhat arbitrary timetables for itself rather than, as Edmund Burke would have urged, by creating the conditions in which rational policies might be expected to evolve. The communique issued in Paris last Saturday says that the nine governments have committed themselves to nothing less than the economic and political unification of Europe by 1980. Those who may have feared that the influence of the British government, with its traditional pragmatism and its transatlantic links, would have worked for a more open-ended approach have thus been confounded, yet a careful reading of the past few months in Britain would have shown quite clearly that Mr Edward Heath was out for nothing less than a united Europe. It is more of a surprise that the government of France should have been able to take the plunge. Does this mean that the old Gaullist conception of a Europe in which member states retain not merely their national identities but their idiosyncratic policies as well has gone for good? Does it imply that the more recent version of the Gaullist conception of Europe, in which France would have been the organizing principle, has also been abandoned?

What the communique has to say about the steps now to be taken towards a united Europe is nevertheless an awkward mixture of declarative policy and sensible pragmatism. On policy towards industry, science and technology, the communique is on the whole pragmatic. It promises that legal and fiscal impediments to company mergers will be eliminated, and that a body of company law will be worked out on a European basis. This is sensible, and indeed overdue, but undoubtedly there will be a great many technical difficulties to be overcome before everybody is satisfied. This is why the European governments should move quickly towards the goal announced last week of opening up public sector purchases to all European companies. One of the continuing obstacles to competitiveness in Europe is the determination of government departments and publicly owned industries to place orders with traditional suppliers, usually national companies. The result is that public purchasing authorities are denied some of the benefits of large scale manufacture and that the suppliers cannot be held to be in competition with each other. An agreement between the nine governments to throw open public sector purchases to all comers within Europe should be comparatively easy to negotiate, but it will also be necessary to ensure that public purchasers such as the telecommunications authorities liberalize their technical standards so as to make competition effective. Nobody should be surprised if that turns out to be a mammoth task, but there is no reason why the job should not be completed by, say, the end of 1973. Moreover, it should be possible to make progress in this direction without

directly involving the Brussels Commission for which everybody should be thankful.

Last week's communique also talks of the definition of common objectives for science and technology and the development of common policies, with the coordination of national policies and institutions. The intention is that "a programme of action together with a precise timetable" should be decided by the beginning of 1974. The most obvious difficulty is that of knowing how to define European objectives for science and technology when national objectives remain only incoherently defined. And to the extent that universities are an essential ingredient of scientific activity throughout the community, it is clear that there cannot be a common European science policy while the present chauvinism of the national university systems persists (see *Nature*, 239, 297; 1972). This is why the commitment to a timetable for coordination by the beginning of 1974 should not be regarded either as a belief that all the problems will be identified, let alone solved, by then or as a licence for the European Commission to formulate yet another over-ambitious plan for the centralization of European technology. Within the next year, however, it should be possible for the community to agree that in due course the national universities should be disestablished, thus making possible the free exchange of teachers (and possibly even of students), and that a start will be made, modestly to begin with, on the European Science Foundation. Between governments, it should also be possible to arrange that publicly supported research in fields such as standards and metrology should be jointly planned and funded—it is absurd that half a dozen European countries at present independently support programmes of research and development in fields like these, and sensible coordination could save substantial sums of money. The question that governments will have to face between now and 1974 is whether they will be prepared to go the whole hog and transfer responsibility for the management of existing standards and metrology laboratories to a common governing body, no doubt a part of the European Commission's appalling bureaucracy. On balance, there is a case for being adventurous in this narrow field. Elsewhere, however, in atomic energy for example, it would be better to look for a close working arrangement between national organizations at present responsible for research and development—the stakes are too high and the failure of Euratom too fresh for the community's procedures to be trusted. In other words, there is much to be said for an understanding that the coming year should be used to develop a variety of machinery for technological collaboration, and for acknowledging in advance that the timetable due to be completed by 1974 should be frequently revised thereafter in the light of experience.

Last week's communique is also too explicit on what it calls environmental policy, where "a programme of action accompanied by a precise timetable" is promised by July 31, next year. Nobody will dissent from the



declaration of the heads of government that particular attention should be paid to 'intangible values and to protecting the environment so that progress may really be put at the service of mankind'. The difficulty is that there is as yet only a meagre understanding of how this should be done. To be sure, there are many opportunities for commonly planned research and development (but few reasons why the rump of Euratom should become the operating agency). To the extent that the most conspicuous forms of environmental nuisance in Europe are still comparatively local problems—the Ruhr and the Rhine, for example—there is also much to be said for encouraging national governments to adopt regulations and controls that will provide their own people with a better life or at least with an opportunity to strike their own economic balance between the cost of getting rid of pollution and the benefits therefrom. But in the long run, environmental policies will reduce to policies on land use—it is surprising that the environmental movement has not tumbled to this simple truth already. So does it not follow that the timetable promised for next July should be less concerned with smoke and DDT than with questions such as whether Europe as a whole is to have a policy on national parks, a policy on the coordinated development of transport links and a common attempt to disentangle the intellectual difficulties of designing cities which are at once efficient and congenial. To promise more is to run the risk of creating disappointment.

The community also needs to think much more about its regional policy. The communique says that the commission will be asked to list the regional problems of the enlarged community and promises that governments will set up a regional development fund before the end of 1973 so as to correct what are called "regional imbalances . . . particularly those resulting from the preponderance of agriculture and from industrial change and structural under-employment". In Britain, Mr Heath will no doubt profit enormously from this part of the Paris agreement, which goes a long way to meet some of the discontent which led the Labour Party's conference, two weeks ago, fatuously to declare its intention of "renegotiating" the Treaty of Rome. The British answer to this question, like that of most other European countries, is that regional policy should aim at offsetting the economic misfortunes which afflict some parts of each country in Europe. If coal-mining dies out in South Wales, if shipbuilding becomes unprofitable on Teesside, or if the south of Italy remains obstinately resistant to attempts at industrialization, then somehow or other extra funds must be channelled into these localities so as to attract new kinds of industry. Yet policies like these are rarely entirely successful. In spite of Mr Harold Wilson's claims for the regional policy of the recent Labour government, people must face the fact that it has proved uncommonly difficult to breathe new industrial life into parts of Britain stricken with accidie. The overriding difficulty is that the cost of industrial location is rarely a sufficiently great proportion of the total cost of operating a manufacturing business for economic incentives provided by governments to be enough to bring work to places where there is not enough. To be sure, it is wrong that particular communities should be allowed to sink into misery simply because local industries have died out, which argues at the very least for generous social services (on which point last week's communique is vague to the point of evasiveness). There

are also strong grounds for doing what can be done to bring work to communities where unemployment is unreasonably high, so long as it is recognized that the results will usually be palliatives, not long-term solutions. As the past thirty years of attempts to prevent the depopulation of the highlands of Scotland have shown clearly enough, communities can learn to live with substantial shifts in the pattern of industry if given time.

Last week's communique is probably as important for its omissions as for what it has to say about the steps immediately to be taken on the road to a united Europe, and the chief omission is defence. Yet it is now apparent that the nine governments must know at least their own minds by the time of the European Security Conference a year from now. On the face of things, it is hard to see how anyone of them can shrink from the question of how best to respond to the impending reduction of American forces in Europe and from the logic that would require a united Europe to have not merely a coordinated defence policy but a common one. No doubt the issue has been delicately left aside until various governments, particularly those of Britain and France, have come to some agreement among themselves. It is hard, however, to think that the next twelve months will go by without some formal agreement on nuclear weapons and on the common planning of conventional defence (see *Nature*, 239, 357; 1972). It would have been sensible if they had acknowledged as much in Paris last week.

## 100 Years Ago



### THE GREAT CIRCUMNAVIGATING EXPLORING EXPEDITION

PREPARATIONS for the expedition which is about to be despatched by the Admiralty for the purpose of dredging, sounding, and otherwise scientifically investigating, the deep sea, have been for some months past in active progress, and are now rapidly approaching completion. The vessel set apart for the purpose is H.M.S. *Challenger*, a main-deck corvette of 2,306 tons, now lying at Sheerness.

It is difficult to over-estimate the immense benefit which science must derive from an expedition such as this. Apart from the results of intense interest which may be expected from the deep-sea work, the principal object of the expedition, and which must go far to elucidate a subject on which our knowledge is at present of the most imperfect description, abundant opportunity will offer for the accurate investigation of the animal and vegetable life of many highly interesting and yet imperfectly known or totally unexplored regions. The investigation of the floras of such islands as Fernando Noronha and the Marion and Crozett groups cannot fail to yield most instructive results; and it is needless to speak of the intense interest which centres in New Guinea.

*From Nature*, 6, 529, October 31, 1872.



## OLD WORLD

# Birth of the Advisory Board for the Research Councils

THE cultural argument for scientific research is, these days, not sufficient to justify the spending of enormous amounts of money on research, according to the third and final report of the Council for Scientific Policy published last week (Cmnd 5117, HMSO, £0.24).

The CSP, which has been wound up as part of the government's decision on science policy last July, is to be replaced next month by the Advisory Board for the Research Councils. For the first year of its existence, the chairman of the board will be Sir Frederick Dainton, chairman of the CSP since 1970. The members of the board include the executive heads of the research councils, senior scientists of user departments and independent members.

In coming to terms with reality the CSP says that it recognises that science must compete for funds with other public needs and that claims for such funds must be judged not "only on their own merits but also in relation to the needs and objectives of society".

Nevertheless, the CSP holds that the planned expansion rate for science in Britain will not be adequate in a few years time to maintain "the same continuing level of scientific activity". The predictions for 1974-75 are for the science budget to increase in real terms over 1973-74 by less than two per cent. This is to be compared with a 4.2 per cent increase in 1972-73 and 13.3 per cent in the halcyon days of 1966-67.

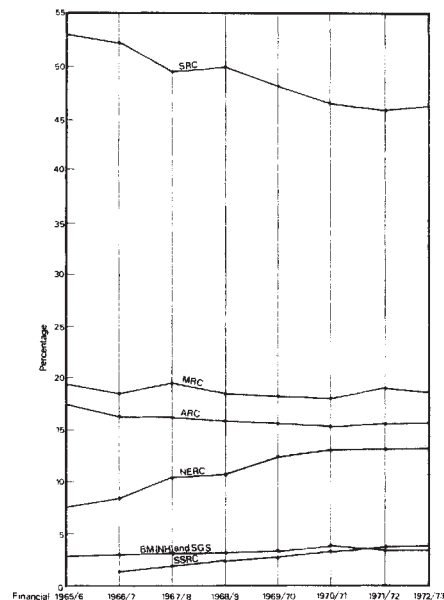
Even though the increased budget is adjusted to account for inflation, it is not automatically adjusted to take into account higher salaries and the increasing cost of building and scientific equipment for which, in particular, increasing sophistication adds costs over and above those allowed by inflation.

The CSP views the prospect of a possible real decrease in the level of support of scientific activity with some distress and it points out that this is likely to occur at a time when the "demands on science are accelerating".

Stated British government policy on the financial support for science in Britain during the next few years is inconsistent with the views of the ministers for science of the OECD countries, says the report. Last year the OECD, which includes Britain, agreed that the 1970s would "require the expansion of research, development and innovation activities to meet social needs such as environmental policy, health, education and urban development". One of the fears of the CSP, unless there is a change in policy, is

that in a few years time important activities will have to be sacrificed with the result that the research councils will not be able to meet pressing demands for new research.

Since the previous report of the council in 1967, the Natural Environment Research Council has received an increasing percentage of the total budget allocated to the research councils (see figure). This, says the CSP, is in accordance with its policy quickly to bring the state of research in the environmental sciences up to that of the other councils. The Social Science Research Council since its inception in 1966 also experienced a rapid increase in its budget during the first few years of its life but that increase has levelled off in the past two years. The CSP says that even more support should be given to the SSRC, especially because more than half its budget goes on



Distribution of the science votes.

## International Chess

THE team from the Soviet Union recorded its eleventh successive victory at the recent biannual Chess Olympiad held in Skopje, Yugoslavia. The team (consisting of Petrosyan, Korchnoy, Smyslov, Tal, Karpov and Savon) was certainly the strongest in the competition since it included three ex-world champions and on paper did not seem to require the services of yet another ex-world champion, Spassky, who was tired after his match with Fischer. Nevertheless, the Soviet Union had rather greater difficulty in fending off its rivals than in earlier years and managed to take the lead only in the later stages of the finals. They succeeded in this largely through the fine form of Tal and Karpov who achieved the best individual scores on their respective boards.

The Hungarian team came second and the Yugoslavs were third. Curiously, this placing of the first three teams was the same as it had been in the previous Olympiad held in Siegen two years ago. It is noticeable that the most successful teams in these events are generally from Eastern Europe.

The first Chess Olympiad was held in London in 1927 when sixteen teams took part, but since the resumption of these events in 1950 there has been a marked increase in the countries eager to participate in them. One result of this expansion has been that the length of the tournament itself has had to be

increased and the Skopje event lasted about four weeks. A second result was the necessity to organize the tournament into sets of preliminary qualifying sections rather than on an all-play-all structure. At Skopje there were eight teams in each section, and only the top two teams from each section assembled to form a "finals" group of sixteen teams.

The United States team came as low as ninth this year due largely to the withdrawal of three of their star players (including Fischer) on the pretext of insufficient financial inducements. Their performance was a far cry from former years—for instance the American team came first in no less than four Olympiads during the 1930s. The Soviet Union, of course, did not compete in these earlier tournaments and has only demonstrated its chess supremacy since the Second World War.

The only players to challenge the dominance of the Eastern European nations at Skopje were the West Germans and they finished fifth in the competition. Their success was chiefly due to their top board, Hubner, who scored the best percentage of all the top boards, although he was rather fortunate to win a game against Petrosyan on the time limit owing to a faulty clock.

The chess Olympiads have retained their popularity over the years as they give the opportunity for all the best players in the world to assemble together under one roof in order to display their particular skills under the guise of national prestige.—J.P.

training research students whose numbers increase every year.

The new Advisory Board for the Research Councils will differ from its predecessor in that many of its members will represent customers and contractors in Lord Rothschild's terms. Its functions are to advise on civil science with particular reference to the research council system and its articulation with the universities and government departments; to advise on national and international activities, to advise on the allocation of the science budget among the research councils and other bodies and to promote liaison between the councils and the users of their research.

#### ANGLO-SOVIET SCIENCE

## Hopes Revived

THE ten-day visit to Britain by a delegation from the Soviet State Committee for Science and Technology which ended last week, sponsored by the DTI and the CBI, will, it is hoped, revitalise the plans for cooperation in science and technology between the two countries first established by agreement in 1968 and reestablished under the Permanent Soviet-UK Intergovernmental Commission for Cooperation in the Fields of Applied Science, Technology, Trade and Economic Relations, which was established in January 1971.

Since September 1971, the scientific and technological activities of this commission have lapsed somewhat. But the visit to London of the Soviet delegation, led by Mr Dmitrii N. Pronskii, head of the foreign relations department of the State Committee for Science and Technology, seems a promising omen for reestablishing the commission.

In January 1971, when the joint commission was established, it took within its scope the working groups on specific technological problems which were established during 1967-68 under the auspices of the CBI and the Soviet State Committee. These had joint chairmen from Britain and the Soviet Union and dealt with cooperation in such fields as the automobile industry, industrial pollution, patents, standards, scientific instruments, medical equipment and instrumentation, information technology and machine tools. A prime purpose of the joint commission, as originally envisaged, was to review annually the work of these groups in the light of the 1968 agreement.

None of the working groups has met for more than a year, however, and some of them have lapsed into what is called a "care and maintenance" basis. Their revitalization, however, would be a matter for the joint commission to discuss when it next meets.

Another possibility for cooperation which has not so much lapsed as never become implemented, is that envisaged by article three of the 1968 agreement, which provides for exchange visits between Britain and the Soviet Union of applied scientists and technologists "to gain experience in industrial research institutes and industrial enterprises". Theoretically, this should supplement the more active arrangement between the Royal Society and Soviet Academy of Sciences, and should provide a framework within which experts not covered by the Royal Society-Academy agreement (which is primarily for "pure" scientists) could gain short-term experience in applied research institutes not belonging to the academy.

In the few cases where exchanges have been arranged within this framework, they have been "self-financing" as far as the British side is concerned, the scientists concerned paying their own fares to Moscow but being fully sponsored by their Russian hosts during their stay. A few visits of Soviet technologists to Britain has also taken place on a similar basis. Clearly, the implementation of the framework of article three on a more regular basis would be greatly welcomed by applied scientists and technologists in both Britain and the Soviet Union, and it is hoped that the joint commission will raise this matter at its next meeting.

The timing of this next meeting (originally intended for January 1972) was one of the chief topics of discussion at the recent talks. Accordingly, the agreement by both sides to reconvene the commission at an early date is a promising sign of future activity. The problem is not, as has been supposed, one of introducing new "machinery" of technological cooperation and exchange, and no such new arrangements have, in fact, been proposed. What is needed is the better implementation of the existing possibilities. The need to discuss these possibilities has been accepted by both countries. It is fairly clear that the next meeting will take place in Moscow, and a tentative date of spring 1973 has emerged from last week's talks.

#### AGRICULTURAL RESEARCH

## Meeting Rothschild

THE Agricultural Research Council (ARC) and the Ministry of Agriculture, Fisheries and Food are to have a joint body to advise them on research and development. The Joint Consultative Organization, as it is known, will replace the ARC's present advisory committees and will advise the council, the ministry and the Department of Agriculture and Fisheries for Scotland on

the scope and scale of all their government financed research and development.

The new organization, which meets the requirements of last July's white paper on government research and development, will link the research council and the government departments more closely than before. It provides for five research and development boards, reporting to the council and the departments, covering animals, arable crops and forage, horticulture, food safety and nutrition technology, and engineering structures. The boards will decide the priorities within their own field, and the detailed consideration of individual projects will be left to research and development committees which will be appointed by the board.

The new arrangements also provide for *ad hoc* working parties which can be set up to investigate specific subjects in depth, and for *ad hoc* ways and means panels to advise the boards and committees on the availability of resources when new projects are proposed.

The Agricultural Research Council was at pains to point out this week that the proposals are still embryonic. The number of committees that will support the boards has yet to be decided and full details of the machinery and membership of the various bodies have not yet been worked out. It is intended, however, that scientific representation on both the research and development boards and committees will be strong, including the directors of the council's and ministry's research institutes and senior scientific staff.

The organization's work will be purely advisory; its recommendations will be passed to the departments and the council which will each decide how to spend its own money. There are no plans for a body directly to coordinate the work of the council and the departments, but it is intended that the secretary of the ARC will be represented on the requirements board of the ministry and that the Chief Scientist of the ministry will be represented on the council. In this way it is hoped that the actual decisions on what research should be supported and to what extent will not result in overlapping between the ARC and the ministry.

Meanwhile, the Ministry of Agriculture, Fisheries and Food seems to have gone a long way to meet the recent criticisms of the Institution of Professional Civil Servants that its new chief scientist would not be master in his own house. The IPCS says it is satisfied with the arrangements now proposed. The ministry says the present arrangements were always those intended—but does so in such a stuffed shirt way that its good sense if not its credibility must be in doubt.



## NEW WORLD

## Academy, Fundamentalists and Soviet Jews

by our Washington Correspondent

A VISIT by a group of scientists from the Soviet Union added a fresh spark of interest and a focus for protest at the autumn meeting of the National Academy of Sciences last week. In other respects, the business meeting of the academy, held in decent secrecy, closely followed the format established during the past three meetings of that august body. One resignation was finally accepted and action on another was deferred. William Shockley was again rebuffed by members of the academy who declined to initiate a study of the basis for his belief that genetic factors make blacks less intelligent than whites, and there was again talk of how the academy should set about getting younger blood into its ranks. Another, and perhaps the most important, action taken during the meeting was the adoption of a resolution urging the California State Board of Education not to adopt regulations which would require special creation to be given equal prominence alongside the theory of evolution in high school textbooks (see *Nature*, 339, 420; 1972). The California education board will consider such a requirement when it meets on November 9.

The group of Soviet scientists, who visited the academy hard on the heels of a group of Chinese doctors, was led by Dr Mstislav Keldysh, President of the Academy of Sciences of the USSR. His presence among the group had been kept secret by the academy, partly because once before when the academy announced that he would arrive he had to call off his visit at the last moment, and partly because of fears of protests about the treatment of Jewish scientists in the Soviet Union. In the event, the meeting was picketed by demonstrators who expressed their distaste for the emigration taxes recently imposed on Soviet citizens who wish to leave for Israel, and Keldysh and his group were also the recipients of some vigorous questioning by academy members during a meeting from which even staff members were excluded.

According to reports of what took place at that meeting, academy members questioned the Soviet scientists about the treatment of scientists in the Soviet Union without specifically mentioning the plight of Soviet Jews, although the implication was clear. The Soviet scientists, for their part, said that they believe that their government's policy has been misrepresented. They

pointed out that scientists in the Soviet Union receive their training from the state, and thus have some obligation to their government—in much the same way that grants and scholarships given to American students often carry obligations concerning employment. Dr Keldysh was due to hold a press conference at the academy last week, but cancelled it because he said he did not know enough about American science. He did promise, however, to hold a press conference before he leaves the United States.

To coincide with Keldysh's visit to the academy, six Soviet Jewish scientists who have been harassed since they applied for permits to emigrate to Israel, telephoned a message to Dr David Korn, professor of Russian studies at Howard University, and asked him to relay the message to Dr Philip Handler, President of the

National Academy of Sciences. The scientists, Dr David Asbel, Dr Alexander Ya Lerner, Dr Benjamin Levich, Dr Boris Moisheson, Dr Roman Rutman and Dr Alexander Voronel, asked Handler to pass on their message to Keldysh and to discuss with him the measures that he must take to protect their basic human rights. Handler passed on the message during dinner with Keldysh last week.

In many respects, the most important action taken at the meeting last week was the passage of a resolution urging the California State Board of Education not to adopt a requirement that creationism be given equal prominence alongside the theory of evolution in school textbooks. Although in most other countries the conflict between religious doctrine and scientific theory, as they relate to the development of man, has long ceased to be a matter of

## NOISE

## Regulating Noise

by our Washington Correspondent

WITH almost its dying gasp, Congress last week sent to President Nixon a bill designed to regulate noise makers in the United States. Dropped from the bill at the last moment, however, was a provision which would have made it virtually impossible for Concorde or any other supersonic aircraft to land at any US airport. The bill had been bogged down in Congress by a fight over whether the Environmental Protection Agency or the Federal Aviation Administration should have the power to set limits on aircraft noise—a bill passed by the House of Representatives earlier this year gave such authority to the FAA, while the Senate wanted to give it to the EPA with FAA retaining a veto on safety matters. There the matter rested until a few hours before Congress adjourned, when final agreement was reached on a formula which essentially gives EPA the power to set regulations, but the FAA will have the last word.

In short, the bill requires the Environmental Protection Agency to draw up noise emission regulations for products such as trucks, automobiles, construction equipment and compressors, and in arriving at the regulations, an assessment of the best

available technology must be taken into account. The EPA will draw up regulations for aircraft which will be subject to public hearings, and after consultation the FAA will be required to accept, reject or modify them and to propose methods of implementing them. The whole process will be open to public hearings and if necessary court proceedings.

As for supersonic aircraft, a provision tacked on to the bill when it came up for debate in the Senate would have required Concorde to meet the noise requirements established for subsonic aircraft. Since, according to Senator Alan Cranston, architect of the amendment, Concorde is expected to make between six and twelve times as much noise as subsonic aircraft, it would have found all US airports closed to it. Although the provision was dropped at the insistence of members of the House, who were prepared to ransom the rest of the bill to get rid of the amendment, it may not yet be a dead letter. For one thing, the EPA in drawing up its regulations will necessarily take into account the likely noise emissions from supersonic aircraft, and its requirements are likely to be as stringent as those for subsonic aircraft. And, for another, Senator Cranston's amendment was adopted in the Senate by such a lopsided margin—62 to 17—that Congress may put it back in next year.

public debate, fundamentalist teachings are still able to hold sway in the United States over scientific thought—the famous debates between Bishop Wilberforce and T. E. Huxley which took place a hundred years ago, notwithstanding. It was only four years ago, for example, that the Supreme Court overruled an Arkansas statute which forbade the teaching of evolution. And now, fundamentalists are hoping for their biggest victory yet in California.

Briefly, the issue started in 1969 when the California State Advisory Committee on Science Education presented a set of guidelines for science teachers to the state education board. The board subsequently complained that creationism was not mentioned in the teaching of evolution and consequently wrote into the guidelines a requirement that special creation be taught in the state schools alongside Darwinism. The guidelines are not mandatory, however, and they have not had much effect. But on November 9 the education board will consider a requirement that all science textbooks used in state schools must give equal prominence to creationism and scientific evolution. The ramifications of such a requirement would extend far beyond the borders of California because the state's schools take about 10 per cent of the textbooks published in the United States and publishers are unlikely to print a special edition just for California.

The academy's resolution states that "the essential procedural foundations of science exclude appeal to supernatural causes as a concept not susceptible to validation by objective criteria; and religion and science are, therefore, separate and mutually exclusive realms of human thought whose presentation in the same context leads to misunderstanding of both scientific theory and religious belief". The resolution adds that "the proposed action (in California) will almost certainly impair the proper segregation of the teaching and understanding of science and religion nationwide". The academy thus urges that "public school science texts be limited to the exposition of scientific matter".

A more straightforward piece of business was the acceptance of the resignation from the academy of Dr Bruce Wallace, a geneticist from Cornell University. Wallace, who sent in his letter of resignation a year ago, is the second member to resign over the issue of the academy's acceptance of work classified as secret. The other was Dr Richard C. Lewontin of Chicago University, who resigned in April 1971, but whose resignation was not accepted until April this year. Wallace's resignation was deferred at the April meeting because the academy then

adopted a resolution relating to classified studies (see *Nature*, **237**, 6; 1972), and members decided to see whether it went far enough to allow him to withdraw his resignation. Wallace said last week, however, that his resignation was "an educational act", designed to demonstrate that Lewontin is not alone in his views on classified studies. Wallace said that his resignation was "a lot less painful than pouring gasoline over myself and setting fire to it".

Another member who also feels the same way as Lewontin and Wallace is Dr Thomas Eisner, also of Cornell University. According to a spokesman for the academy, Eisner has sent in a letter of resignation which included a few points of inquiry. Handler answered the points, but because no further communication has been received from Eisner, who is now on a year's sabbatical in Australia, it was decided at the meeting last week to defer action on his resignation until the annual meeting next spring. The academy has a peculiar rule which does not allow a member officially to resign until his resignation has been accepted by the membership.

#### WATER POLLUTION

### Veto Overridden

by our Washington Correspondent  
A SHORT time ago, when campaigning in California, President Nixon castigated the Democrat-controlled Congress for sitting on the Administration's environmental legislation. "The members of the Senate and the House are simply not keeping pace with the concern of citizens throughout the nation for positive action" against pollution, he declared. A couple of weeks later, however, Congress sent him a far-reaching bill designed to clean up rivers, lakes and streams in the United States, and he vetoed it. Immediately, however, both the House and the Senate easily overrode the veto and in President Nixon's words, "rammed (the bill) into law over the better judgment of the Executive".

The water pollution control bill, which has been nearly two years making its way through the committees and lobbies of Capitol Hill, sets as its goal the elimination of the input of all pollutants into America's waters by 1985—they should be clean enough to swim in by 1983. As an interim goal, the bill sets 1977 as the target date by which all municipal and industrial dischargers should have installed the "best practicable" control technology. Although President Nixon made clear in his veto message that he does not disagree with the bill's "laudable intent", he does disagree with the expenditures it would entail—\$24,000 million to be earmarked over the next three years,

although the spending would be spread out over a longer period.

The central point of President Nixon's veto message is that if the bill becomes law—as indeed it has—one inevitable result will be a tax increase. But the bill does allow the Administration considerable flexibility in deciding just how much of the money should be spent, and Nixon made clear last week that if he is re-elected he will "use those provisions to put the brakes on budget-wrecking expenditures as much as possible". In particular, President Nixon pointed out that the Administration had originally proposed a bill which would have entailed expenditures of only \$6,000 million.

Briefly, the bill provides the Environmental Protection Agency with authority to let contracts for construction of new waste treatment facilities by state and local governments. The total funding over the next three years for this provision is \$18,000 million, with the federal government contributing 75 per cent. A minimum of secondary treatment is required for all projects started before 1974, after which the criterion of "best practicable control technology" must be employed. Similarly, industrial polluters would be required to employ best practicable control methods by 1977.

The second phase of the attack on water pollution would take place between 1977 and 1983, when industries and municipalities would be required to move from the "best practicable" to the "best available" control technologies, with the goal of eliminating all discharges by 1985. This goal is, however, not legally binding. In addition, EPA is required to survey the waters in the United States so as to identify by the end of 1973 those waterways which already satisfy the 1983 water quality goals and those which will satisfy the goal by 1977 and by 1983.

Clearly, one of the central requirements of the bill is the determination of what constitutes the "best available" or the "best practicable" technology. The requirement for the best practical technology will be determined by the Administrator of the EPA, in the light of such factors as the financial ability of companies to comply, the effect on employment and on the economy, while the more stringent requirements for the second phase of the attack on water pollution will be defined by a 15-member commission whose members will be appointed in equal numbers by the President, the Senate and the House of Representatives.

A few days before the bill was vetoed by President Nixon, William Ruckelshaus, Administrator of the EPA, wrote to Caspar Weinberger, Director of the Office of Management and Budget, urging that the bill be signed. Ruckelshaus



haus based his advice on the fact that not all the money entailed in the bill need be spent, and also that it can be strung out over several years to minimize the impact on the budget. Nixon preferred, however, to stick to his principle of trying to hold down taxes and overrode Ruckleshaus' advice. Now that Congress has got its way, the Cuyahoga River and the Houston Ship Canal—both of which have caught fire recently—may become recreation areas by the late 1980s.

#### LABORATORY ANIMALS

## A Plague for Frogs?

by our Washington Correspondent

A CURIOUS and so far unexplained ailment which killed off millions of frogs in the United States last winter and which seriously curtailed supplies of the animals for teaching and research, may be showing itself again this year. A year ago, when dealers began collecting frogs just before the hibernation season, many of them found that their animals died within a few weeks. But the phenomenon was not confined to frogs in captivity: rows of the dead animals were found in collecting ponds when the ice thawed in the Spring.

The effect of this widespread mortality was such that some supplies of frogs for teaching and embryological research dried up last winter, and preliminary indications are that the same process may be repeating itself. A large dealer in the Midwest said last week, for example, that he has already stopped buying frogs from Minnesota, which has previously been his chief source of supply, because none collected during the past week or two has survived.

But another large Midwestern dealer gave a more optimistic view of the situation. He said that he already has good frogs in hibernation which should be sufficient to meet all the demand this winter. The problem, he suggested, is confined only to certain areas in some of the midwestern states, because he has supplies from Canada, North Dakota, Minnesota and Wisconsin, and has found that with good care the frogs have gone normally into hibernation. Each year some 20 million frogs are used for teaching in the United States and about 2 million for research.

The symptoms of the ailment are that the frogs redden, which may be an indication of bacterial infection, and then die. Opinions differ on the cause of the phenomenon and so far it is still a mystery. But the United States is not the only country that is having problems with frog populations, which have been almost wiped out in parts of Great Britain during the past few years.

One theory that has been put forward is that 1970 was a particularly good spawning season, but that in the long dry summer of 1971, aquatic food was hard to find so that many animals went into hibernation in a state of malnutrition and succumbed more easily to bacterial infection. The symptoms of the ailment seem to indicate that infection of some sort is involved, but it has also been suggested that agricultural chemicals may have done the damage.

A buyer from one of the midwestern dealers pointed out last week, for example, that heavy rains fell last autumn, and again this year, increasing agricultural runoff just at the time when the frogs entered the waters to hibernate. But he said the chief evidence to implicate agricultural chemicals comes from his finding that frogs caught in September, before they entered the ponds, have hibernated normally and seem to be quite healthy, while those caught in October have died. Moreover, at a storage facility in Minnesota last year, a batch of frogs stored in hibernation in well water survived, while another batch stored in lake water died. So far, however, analyses of lake water samples have failed to turn up evidence of excessive amounts of agricultural chemicals and in any case the bacterial infection itself could have come from the lake waters.

#### ELECTION 1972

## Nixon's Friends

by our Washington Correspondent

WITH just three weeks to go to the election, a group of scientists and engineers has been formed to work for the reelection of President Nixon. Called the Science and Engineering Council in Support of the President, the group will work under the chairmanship of Dr William O. Baker, vice-president of Bell Laboratories Inc. and former boss of Dr Edward David, the President's Science Adviser. Although the council will initially take its cue from the Committee for the Reelection of the President, it was made clear last week that its presence may be felt long after November 7.

The council has entered the campaign very late in the day, and several weeks after a committee of scientists and engineers started working for Senator McGovern. But the council will be more than just a tool in the election campaign, for Dr Baker said last week that if President Nixon is reelected, it will continue to function as an independent source of advice to the Administration.

Although such functions are already being performed by a variety of organ-

izations, including the Office of Science and Technology, the President's Science Advisory Committee and the National Science Board, Baker pointed out that the council's chief value would be its independence and—given that its members hold exalted positions in the scientific community notwithstanding—its ability to represent grassroots thinking. Baker added that the council is not expected to receive formal financing after the election.

So far, science and technology have not become issues in the campaign, and Baker acknowledged that there is little disagreement between the Republicans and the Democrats on the issues involving science and technology—both party platforms, for example, emphasise the need for using science and technology to help solve domestic problems. But the council has been formed to tell the American public "on behalf of scientists and engineers, the opportunities of continuing the historic commitment of the last four years in the beneficial use of scientific and technical education, research and development". When the Democrats have mentioned science and technology, however, they have suggested that the Administration's rhetoric has not been matched with dollars, and that some of the money being spent on defence and space research and development could more profitably be spent on domestic projects.

As for unemployment among scientists and engineers, Baker said last week that the situation has been "very greatly relieved". He said that the council thus supports the Administration's view that special measures to relieve unemployment among scientists and engineers are no longer needed. But Dr Vigdor Tepitz, Secretary and Treasurer of the Scientists for McGovern Committee, commented last week that his committee has found unemployment to be still a major issue among members of the scientific community and that it has not yet been solved.

Baker said last week that the council of scientists for Nixon would work chiefly by responding to requests for position papers and other matters directed to it by the Committee for the Reelection of the President. Scientists for McGovern, on the other hand, have been concentrating on canvassing the scientific community by mail and it has also contributed to the position papers delivered by McGovern on the environment and energy.

Included among the 29-member council formed last week are Dr Simon Ramo, Dr Robert Charpie, Dr Lawrence Goldmuntz, Professor Willard Libby, Professor Richard Morse, Dr William Nierenberg, Professor Bernard Oliver, Professor S. Fred Singer, Dr Athelstan Spilhaus and Dr Edward Teller.



# Physics and Chemistry Nobel Prizes

AMERICANS have made a clean sweep of this year's Nobel Prizes in chemistry and physics which were announced last week. The physics prize is awarded for the formulation of the BCS theory, aptly named after its originators, to John Bardeen of the University of Illinois, Leon N. Cooper of Brown University, Rhode Island and J. Robert Schrieffer of the University of Pennsylvania. BCS theory gives a macroscopic description of the properties of superconductors. One half of the chemistry prize is awarded to Christian B. Anfinsen of the National Institutes of Health at Bethesda, Maryland and the other half is divided between Stanford Moore and William H. Stein of the Rockefeller University, New York, for their work which led to the determination of the composition and the action of the enzyme ribonuclease.

Professor John Bardeen becomes the first person to win two Nobel Prizes in the same category. He is not, however, the first person to win two Nobel Prizes, that honour falling to Marie Curie who was awarded the physics prize in 1903 jointly with her husband, Pierre Curie, and H. Becquerel, and the chemistry prize, eight years later, was awarded to her alone. Linus Pauling has, of course, also won two. John Bardeen was awarded his first prize in 1956 jointly with W. Shockley and W. H. Brattain for their discovery of the transistor effect.

The award of the physics prize to Bardeen, Cooper and Schrieffer marks the end of one of the most exciting hunts in science in this century. Superconductors, the first of which was discovered in 1911, are metals which lose their resistance to direct current at very low temperatures, mostly below 15 K. The understanding of this property presented a problem for physicists that many leading theoretical physicists have attempted to solve. In 1956, Cooper, then a research associate at the University of Illinois, pointed out that two electrons in a metal with energies near the Fermi energy which weakly attracted each other would form a resonant state, now called a Cooper pair. One Cooper pair, however, does not make a superconductor. It was a year later that Bardeen, Cooper and Schrieffer (then a research student) working together at the University of Illinois, showed how Cooper's idea could be applied to many electrons and how a new cooperative state of all the conduction electrons, the super-



Professor John Bardeen

conductive state, would be formed.

The publication of this theory has given rise to a long period of fruitful research, which confirmed and extended the original work. For instance, B. D. Josephson used the theory to predict a variety of microscopic quantum phenomena and these, in turn, have led to new sensitive devices for measuring electric currents, voltages and magnetic fields and to the new international standard for the volt. As yet, there have been no everyday applications of superconductors but there remain the possibilities of their use in underground electric transmission lines.

The award to Anfinsen, Moore and Stein will meet with the approval of all protein chemists, for nobody has done more in these past twenty years to determine the shape of the field as we know it today. The names of Moore and Stein are inseparably linked (to the point where many students of biochemistry must have wondered whether they had any independent existences) in a series of discoveries. They were responsible in the first place for developing the technique of amino-acid analysis, which cleared the way to the determination of protein sequences. They recognized at an early stage the potentialities of ion-exchange chromatography as a means of fractionating so complex a mixture of species as the amino-acids in a protein hydrolysate, and many of the associated techniques that are now taken for granted were evolved by the painstaking chemistry of Moore and Stein. The culmination of their work at this stage was the automatic amino-acid analyser, which is

now to be found in every biochemistry laboratory. Moore and Stein, with some of their younger associates at the Rockefeller Institute, developed methods of labelling and identifying residues at catalytic sites, and succeeded in defining the constituent side chains in that of ribonuclease. In what was at the time a great technical *tour de force*, they determined the total sequence of this chain of 124 residues.

Anfinsen's name is also to a considerable extent associated with pancreatic ribonuclease, for it was with this enzyme that he first demonstrated the principle that the three-dimensional structure of a protein is uniquely determined by its sequence. He showed that in proteins that contain disulphide bonds, the refolding process occurs by a continual shuffling of the many possible pairings until the pairing that corresponds to the native conformation is attained. He also found that molecules containing incorrect pairings could be isolated, and that they would in time, under mild oxidizing conditions, rearrange to give the native disulphide arrangement and conformation. Anfinsen and his colleagues next looked for and found a protein in animal tissues that catalyses disulphide exchange, and so greatly hastens the rate of appearance of the active conformation of enzymes. Anfinsen's interest in folding and its relation to the newly synthesized chain led him also to study the synthesis of ovalbumin in oviduct tissue, and the resulting publications gave important information on the rates of the processes that were involved, and stand as among the most elegantly conceived and executed essays in the field of protein synthesis. Another of Anfinsen's objectives was to synthesize the enzyme *ab initio*, and this has been to a considerable extent achieved, for it was found that the molecule could be non-covalently assembled (like the ribonuclease S-protein-S-peptide system of Richards) from fragments. This discovery made possible a series of elegant experiments on the factors that determine the folding, by changing or omitting individual side chains, and by looking at re-combinations of overlapping sequences. A by-product of this and other work in Anfinsen's laboratory has been a series of important contributions to the technique of affinity chromatography, an approach that has now achieved a position of central importance in hormone research and other areas.

## NEWS AND VIEWS

## First Observations from Copernicus

THE first observations from the orbiting astronomical observatory Copernicus were reported in *Nature Physical Science* last week (239, 135; 1972). The X-ray star Cygnus X-3 was observed on September 7, 19 and 29 by the instrument provided by the Mullard Space Science Laboratory (MSSL). Unlike the radio observations the X-ray data do not indicate any great enhancement in the flux but do show clear evidence of periodic variability on the time scale of a few hours. There is already speculation that the radio emission may not come from the X-ray object.

The X-ray telescopes on Copernicus, proposed by Professor Robert Boyd in 1963, represent the first use of focusing X-ray optics in an orbiting observatory for non-solar X-ray astronomy. Earlier X-ray instruments on satellites have been of the survey type in which detectors of large area, with mechanical collimators, scan as the satellite rotates. NASA's Uhuru satellite has catalogued some 130 X-ray objects in this way.

Copernicus has arrived at a timely moment in the development of X-ray astronomy, for more detailed studies of the sources listed in the Uhuru catalogue are now needed. The position and mapping of many of the sources require considerable improvement, and spectra need to be measured over a wide range. And, of course, long and short term measurements of variability can give an insight into the type of X-ray source. The instrument aboard Copernicus, with its small acceptance angle, wide wavelength range and ability to point for extended periods, can tackle all these problems.

The X-ray instrument has prime viewing time one day in every ten. Princeton University's ultraviolet telescope occupies 90 per cent of the time with an extensive programme of high resolution spectroscopy of bright ultraviolet stars and interstellar matter. Both X-ray and ultraviolet instruments view in the same direction, and simultaneous operation has proved feasible and easy to plan. If there are X-ray emissions from the Princeton ultraviolet targets, they can be detected to limits lower than those set by previous measurements. The desirability of observing the X-ray isotropic background and its fluctuations provides an additional stimulus for joint operation of the instruments.

The limited prime observing time for known X-ray targets will allow study of thirty or so sources a year and viewing priorities must be established. A panel, chaired by an MSSL project scientist, is to assess proposed observations; it includes representatives of the optical, infrared and radio astronomy communities in Britain. Observations have been proposed by astronomers from MSSL, the University of Leicester, the University of Birmingham, the University of Cambridge, the Royal Greenwich Observatory, the University of Sussex and Jodrell Bank, and the wide participation in the observing programme should benefit collaboration in British astronomy.

The faultless performance of the systems aboard the Copernicus spacecraft and the extent by which they exceed specification is quite remarkable. For example,

the pointing jitter of 0.03 arc s, when viewing bright ultraviolet stars, is three times lower than specified and it will inevitably improve the quality of the photometry. Hardly any X-ray targets have bright optical counterparts so that in this case pointing has to be under the control of an inertial reference unit. The drift of this unit is 2 arc s an hour, which enables lengthy observations or scans of an object to be made before time has to be spent updating the reference unit with an observation of a bright star. The indications are that X-ray objects can be positioned to 20 arc s when the pointing of the instrument is fully calibrated.

Copernicus is demonstrating that a large space observatory with excellent pointing capability is now technically possible. Unfortunately it will be the next decade, perhaps, before a large observatory devoted to X-ray astronomy is in orbit. In the meantime there are plans for more sensitive survey missions in several national programmes, so that by the advent of the large observatory the number of X-ray sources may well exceed 1,000. Most of these additional sources will be extragalactic. There is a clear need for additional X-ray missions, before the large observatory is launched, with telescopes at least ten times larger than those on Copernicus. It is encouraging that the ESRO project of a highly eccentric lunar occultation observatory received wide support at a recent colloquium, for it is the kind of mission ideally suited to more detailed studies on faint X-ray sources.—From a Correspondent.

## Proteins and Fused Cells

FOR many years biochemists have wrestled with the problem of identifying proteins within single cells. Anyone reared on the standard histological procedures soon realized how inadequate and unspecific most of the staining methods were. Moreover the urge to identify the individual protein types within a cell has intensified under the pressure of theories about gene expression, differentiation and clonal selection.

Because most cells possess many thousands of protein types at any one time, attempts to study the protein contents of single cells have concentrated on the red blood cell, possessing as it does few other proteins besides haemoglobin, which is often present in more than one form. So biochemists and cytologists have addressed themselves to the task of identifying the particular haemoglobin species within individual red blood cells. To date four methods have been tried. First, blood cells have been challenged with fluorescently tagged antibody raised against the haemoglobin, different colours of label being tagged to the antibody against the differing haemoglobins. Such an approach has been exploited by a number of workers such as Dan and Hagiwara (*Exp. Cell Res.*, 46, 596; 1967) and Maclean and Jurd (*J. Cell Sci.*, 9, 509; 1971). A second method has been used by Gitlin *et al.* (*Blood*, 32, 796; 1968), in which blood cells



are gently lysed and their contents electrophoresed in the presence of antihaemoglobin antisera. The third approach, used by Matioli and Niewisch (*Science*, **150**, 1824; 1965) involves electrophoresis of red blood cells in a bed of agar and identification of the emerging bands of haemoglobin by the use of light at 410 nm. A fourth approach, and certainly a better one, was adopted by Rosenberg (*Proc. US Nat. Acad. Sci.*, **67**, 32; 1970; she succeeded in separating the constituent haemoglobin of single red blood cells on very fine threads of polyacrylamide gel.

In this issue of *Nature* (page 520), Rosenberg has coupled her fine detection system to the technique of fusing cells by exposure to Sendai virus, the method so usefully exploited by Henry Harris and others to form artificial heterokaryons. She has formed heterokaryons by fusing the erythrocytes of bullfrog tadpoles with those of the froglets of the same species. The haemoglobins synthesized by the tadpole and frog differ, and can be separated easily by conventional polyacrylamide electrophoresis. Very wisely, Rosenberg has compared the frog-tadpole fused cell with dikaryons of frog-frog and tadpole-tadpole, exposing individual dikaryons in a medium containing  $^{14}\text{C}$ -leucine for 3 h, and then, following their individual lysis in distilled water, has electrophoresed the haemoglobins on polyacrylamide threads. By the use of the haemoglobin-specific stain benzidine (a potent carcinogen incidentally) the tadpole and adult haemoglobin bands are identified on the gel threads, cut out, and their radioactivity assayed by scintillation counting.

Rosenberg's communication is essentially a very preliminary report of an extremely promising combination of techniques. Excitingly enough, even at this stage in the game, she offers some ideas to play with. For the radioactive counts suggest that the heterokaryons make more frog haemoglobin than the frog-frog dikaryons, and less tadpole haemoglobin than the tadpole-tadpole dikaryons. A similar result, she says, has recently been obtained in cell-free haemoglobin synthesizing systems from *Rana catesbiana* frog and tadpole erythrocytes. Rosenberg hints at her inclination to interpret these results in terms of altered genetic expression in the heterokaryons, the frog cytoplasm derepressing the frog haemoglobin cistrons and repressing the cistron for tadpole haemoglobin. There is reasonable evidence from other work that the haemoglobins from the bullfrog adult and tadpole share no common globin chain.

Interestingly, Henry Harris has interpreted his evidence regarding haemoglobin synthesis in heterokaryons of mouse fibroblasts and chicken erythrocytes in a similar way. When chicken erythrocytes were fused with mouse fibroblasts by exposure to Sendai virus, two chicken-specific proteins were detected as synthetic products in the heterokaryon, the one a chicken cell surface antigen, and the other a soluble enzyme, inosinic acid pyrophosphorylase. (The mouse cells used in the experiment were derived from a mouse strain which failed to make this protein.) But curiously enough, although there is an initial burst of haemoglobin synthesis in these cell fusions, this wanes after some hours, and eventually haemoglobin synthesis can no longer be detected. Harris has suggested that repressor molecules, directed against the globin cistrons and active in the mouse fibroblasts, may be responsible for shutting down the expression of the chicken erythrocyte globin genes.

But caution must be exercised. Rosenberg has no evidence to offer as yet that a genetic regulatory switch is involved. Indeed, a more simple explanation in terms of effective pools of amino-acids or transfer RNA molecules could easily account for her present findings that synthesis of frog haemoglobin is enhanced and tadpole haemoglobin retarded in the heterokaryon.

Two further comments about this interesting communication seem justified. First, it is not clear how the heterokaryons were identified. Was it possible to separate them from the homokaryons, which presumably also form in the mixed cell suspension, by cytological means, or are they identified by hindsight after the electrophoresis reveals two haemoglobin bands?

And second, the levels of labelling detected in the double cells by scintillation counting seem surprising; the assay detected up to 150 counts a minute in the haemoglobin bands emerging from only one fused cell. As the total  $^{14}\text{C}$  used was  $10\text{ }\mu\text{Ci}$  and, by definition, this will yield  $22 \times 10^6$  disintegrations a minute, the utilization of this label by what must be some thousands, if not millions of erythrocytes, can leave little to spare for the individual cell. No doubt Miss Rosenberg knows her way round this particular equation. A fuller report of her experiments is awaited with great interest.—N. M.

## What is Random Packing?

"WHILE many mathematicians believe, and all physicists know, that the density (of packed spheres) cannot exceed  $\pi/\sqrt{18}=0.7404\dots$ "

Thus C. A. Rogers introduced his classic paper of 1958 on rigorous upper bounds to the possible density of sphere packings; he went on to prove, beyond all mathematical doubt, that, if some packing more dense than the close-packed lattice should ever be achieved, then this would certainly have density less than  $\rho=\sqrt{18}(\arccos 1/3-\pi/3)=0.7797\dots$ . This proof, as elegant as it is unconstructive, still stands today as the best available bound in three dimensions, neither confirming nor denying the possibility that irregular arrangements denser than the close-packed lattice may still exist in the range  $0.7404\dots < \rho < 0.7797\dots$

The working practitioner in statistical mechanics can hardly fail to be at once amused and exasperated by the gulf between the austere and quite unhelpful mastery of the geometers on one hand and, on the other, the strange ingenuities of the practical packers and counters, whose experiments have become almost a craze in the past ten years or so. Yet few of the latter, one imagines, would now be willing to bet much of their research budget on the possibility of finding a random structure which improves on the close-packed lattice density. Ball bearings and similar objects have been shaken, settled in oil, stuck with enamel paint, kneaded inside rubber balloons—and all with no better result than a density of about  $\rho=0.636$ .

Wiping their hands after these attempts, the authors concerned may well take comfort in the fact that the theoretical problem is at least as old as Gregory's conjecture (can thirteen spheres be arranged in contact with a single one?) while experiments in practical packings go back to the early botanists such as Stephen Hales (1727). In any case there is now far more to the problem

than attempts to set a record for the highest density, and this aspect has tended to lose prominence in the most recent work. Even the present upsurge of activity in what one might call "applied random geometry", although undoubtedly stimulated by Bernal's perception of the intimate relation between random structures and the fluidity of liquids, is not quite as new as is sometimes thought. Rice, for example (*J. Chem. Phys.*, **12**, 1; 1944), discussed the statistical mechanics of dense hard-sphere fluids in some detail and drew upon the early and inaccurate packing experiments of the ceramicists Westmann and Hurill (*J. Amer. Ceram. Soc.*, **13**, 767; 1930). It is certainly the followers of Bernal, however, who have set the tone for later work and have given urgency to the central task of defining precisely what it is that we mean by a random packing of objects, a problem in many respects far more interesting than that of settling bounds to the attainable density.

Happily it is now no longer necessary to shake large assemblies of balls in containers, with elaborate precautions to prevent them taking up regular arrangements at the boundaries. An ingenious computer programmer can now simulate all manner of packed assemblies, with and without the influence of gravity and with as many balls as his conscience and the computer centre will allow. An unusual example of this type of work appears in this issue of *Nature* (page 504). Visscher and Bolsterli of the Los Alamos Laboratory present detailed computer simulations of several packed systems in both two and three dimensions, including a number of studies with spheres and disks of grossly or slightly unequal size. The packing of unequal spheres introduces some interesting features not present in the mono-size problem. The addition of a proportion of small spheres will obviously tend to give a more dense packing because of their ability to fill the interstices between the larger ones, but there is evidently a limit to this when the spheres become extremely small and numerous. The Los Alamos work indicates that the maximum possible density is probably obtained only with spheres having a disparity in diameter of some hundreds or thousands to one.

A second group of results shows very clearly the tendency for two-

dimensional packings to exhibit a domain structure provided that edge effects can be removed. A careful search of corresponding three-dimensional structures, however, reveals no evidence at all for the occurrence of domains.

Computer studies of packing problems are interesting not only for their cleanliness and convenience but because they enable different algorithms for the construction of assemblies to be tested and compared. The algorithm used by Visscher and Bolsterli is a "physical" one involving the "rolling" of balls or disks into minimal positions under simulated "gravity"; other authors, for example Adams and Matheson of the University of Essex (*J. Chem. Phys.*, **56**, 1989; 1972), prefer to add successive atoms at surface facets closest to a growth centre; still others have filled a region of space with random points, then allowed these to expand into spheres, which then nudge each other apart until the whole aggregate locks solid. This last is the basis of the best algorithmic definition of random close packing, due to Stillinger, DiMarzio and Kornegay (*J. Chem. Phys.*, **40**, 1564; 1964). Nevertheless, none of these prescriptions appear to reproduce very well the precise packings obtainable in mechanical experiments. Visscher and Bolsterli attempt to write an extended algorithm to provide for computer "shaking" of the bin, but the improvement obtained in this way is marginal. One should perhaps comment here that to design a program to simulate the complicated  $N$ -body effects present in "shaking" might well involve a problem of a higher order still, on the level of a good theory of the propagation of sound and temperature waves in the fluid.

Yet, whatever the magnitude of these difficulties, there can be no doubt that the problem of sphere packing has taken on something of a new lease of life with the advent of computer methods. At the same time, the prospects for theoretical advance do not seem to lie so much in the search for Rogers-type bounds—after all a rigorous proof that the close-packed lattice is a bound to the density would only remove a rather forlorn hope of improving on this without necessarily penetrating the nature of randomness in less dense structures.

What can hardly be denied after the recent studies is that the random close-packed aggregate of  $\rho \approx 0.637$  . . . has a unique fascination about it. Can this uniqueness be defined through some statistical geometric determinant rather than simply a packing algorithm? Can some geometrical factor be found which has an extremum at this point, from which an extremum in density follows? What is the relation between the random-packing density and the behaviour of the hard-sphere fluid as treated by conventional statistical mechanics? As Lefevre has recently pointed out (*Nature*, **235**, 20; 1972) there does seem to be a consistency with the results for computer simulated hard-sphere gases at finite temperature. For when data from various Monte Carlo and molecular-dynamic studies are extrapolated to the point of zero compressibility, what should be found but a density neither that of the close-packed crystal, nor that predicted by popular equations of state—but the familiar figure of  $\rho \approx 0.64$  . . . ? If this is more than pure coincidence, it surely expresses the simple fact that thermal agitation at finite temperature is as good—although not significantly better—a "shaker" of spheres as the complicated mechanical devices used in the laboratory. Evidently the thermodynamic system seems to know where it is going as it compresses to the glassy state, even if we remain theoretically little the wiser as to what that state really is.—From our Molecular Physics Correspondent.

## SOUTHERN HEMISPHERE ASTRONOMY **Schmidt Survey Plans**

by our Cosmology Correspondent

THE prompt completion of the 48-inch Schmidt telescope at Siding Spring, Australia, and close collaboration with the European Southern Observatory (ESO) seem likely to result in a complete two colour survey of the part of the southern sky so far unmapped (below  $-30^\circ$ ) within three years from now. At a recent specialist meeting of the Royal Astronomical Society, plans for this collaborative survey were described, and a call for proposals of other tasks for the 48-inch Schmidt was made.

Professor V. C. Reddish (University of Edinburgh) described the telescope, its building and the site, adjacent to that of the Anglo-Australian 150-inch instrument. The telescope itself is a deliberate copy of the Palomar Schmidt which produced the familiar Palomar Sky



Survey; Professor Reddish emphasized, however, that technological developments in the decades since that telescope was built have made possible considerable detailed improvements in the new telescope. For example, the tolerance of focus is much better than that of the Palomar Schmidt, and the new machine is guided by a sophisticated combination of digital and analogue computers which makes operation a one-man push-button affair.

The target date for completion of the instrument is July 1, 1973, just two years after the signing of the contract for its construction. This time scale is only half that which has been required for any comparable telescope, but construction is going ahead well on schedule at present. Although the ESO Schmidt will become operational rather earlier next year, there is certainly enough work for two such instruments in the southern hemisphere. There is already close collaboration between British and ESO groups, who have planned a joint programme of surveying.

First, the ESO Schmidt will be used for what Professor Reddish terms a "quick and nasty" survey, intended primarily to help radio astronomers to identify their sources. This will take less than a year. Starting at the same time, but taking two years to complete, the ESO group will survey the southern hemisphere below  $-20^\circ$  using red ( $H\alpha$ ) plates and fields centred  $5^\circ$  apart. This will begin in January 1973, and by September 1973 the British Schmidt will be surveying the same fields in the blue (using IIIaJ emulsion).

The Palomar Sky Survey extends to  $-30^\circ$ , so that there will be two  $5^\circ$  steps of overlap with the southern survey. Although the ESO Schmidt will produce smaller plates than the new 48-inch instrument, these will have the same scale and will be centred on the same fields—the 48-inch plates will have more overlap, but no astronomer will complain about that. Although the ESO group has the advantage of starting first, the SRC IIIaJ survey will extend one magnitude further, to 22 mag. At present there seems no reason to believe that either group will attempt to use the advantages of their own instrument to steal a march on the other, first because both groups do indeed have certain advantages to trade off, and second because a happy cooperative atmosphere has been firmly established before surveying has begun.

Undoubtedly, the sky survey will be the prime task of the 48-inch at first, and will fill a serious gap in observations. But this can only be carried out during the dark of the Moon, and it is also planned to allocate 10 per cent of this prime time to other projects. That leaves 60 per cent of the available telescope time to non-survey work—much

of it will have to be carried out during light or grey of the Moon, but that is not an impossible handicap for many projects. The second purpose of this recent meeting, then, was to ask for suggestions concerning the use of the non-survey time which will be available. Some of the proposals already being considered include searches to find stars within 20 pc of the Sun and identifications of radio sources with distant galaxies.

It certainly seems that the British Schmidt at Siding Spring will have a full and active life; a note of caution was, however, introduced by Dr M. Rowan-Robinson (Queen Mary College) who urged that a programme of calibrations should be considered urgently as an integral part of the survey, because otherwise the plates will be useless for many purposes until such calibrations of reference sources have been carried out. This note of discord was, however, dismissed in the general euphoria as only a minor detail. The chief "complaint" apart from this came from the radio astronomers; Dr Denis Walsh (Jodrell Bank) lamented the fact that no comparable instrument is available to them in the northern hemisphere—the advantage of the extra couple of magnitudes over the Palomar Sky Survey would be considerable.

#### COLLAGEN

### The Skin Game

from our Molecular Biology Correspondent  
COLLAGEN is probably the most abundant of all proteins: Piez gives  $10^{12}$  kg as a conservative estimate for the amount spread around the world. This alone is sufficient reason for taking an interest in its structure, concerning which a vast amount is already known. Nonetheless, it tends to be regarded as

a rarefied field, which outsiders do not often penetrate. The general features of structure, from the sequence to the fibril, are established, and, apart from biosynthesis and assembly, most of the activity is now directed towards filling in the chemical detail, on comparing the collagens of different species and tissues, and on determining the basis of pathological defects.

Most collagens contain two types of polypeptide chain,  $\alpha_1$  and  $\alpha_2$ , in the mole ratio of 2:1. Each molecule is a characteristic three-stranded rope with, depending only slightly on the origin, about 1,050 amino-acids a chain. Throughout most of the sequence every third residue is glycine, followed commonly by proline, and often preceded by hydroxyproline. This regularity is missing only at both ends, which are also of particular interest because it is here that the covalent cross-links occur. Large parts of both the  $\alpha_1$  and  $\alpha_2$  chains have been sequenced, and the various tracts are indexed along the chains in terms of the fragments that are generated by cyanogen bromide cleavage at the few methionine residues. The  $\alpha_1$  chain gives rise to nine and the  $\alpha_2$  to six such fragments.

Balian *et al.* (*Biochemistry*, **11**, 3798; 1972) have now completed the sequence of one of the two longest cyanogen bromide fragments (termed CB1) of the  $\alpha_1$  chain of rat collagen. This fragment, as Bornstein found, contained a labile asn-gly bond, which could be cleaved by hydroxylamine to give pieces of 99 and 180 residues. It is the second of these whose sequence is now also reported. Because CB8 comes from the interior of the  $\alpha_1$  chain, the triplet repeat is maintained, and only once in 279 residues does glycine occur anywhere except in the first position in each triplet. There are well defined preferences amongst the other amino-acids for the second or third

### Right Side Outside

In next Wednesday's issue of *Nature New Biology* (November 1), Kant and Steck put right earlier erroneous conclusions drawn from work on inside-out vesicles of red cell membranes. Sealed vesicles, as has long been recognized, can be prepared from red cell ghosts in such a way that the outer surface is the inner surface of the original membrane. These vesicles, as Kant and Steck show, can be completely sealed, so as not to pass sodium or potassium ions.

They use sedimentation in a dextran gradient to separate fractions, which they identify as sealed and unsealed vesicles. The sealed vesicles are tested for availability of acetylcholinesterase and diaphorase activity, these being confined respectively to the outer and inner surfaces of the red cell. Thus the right-side-out vesicles give a positive acetyl-

cholinesterase response, whereas the inside-out vesicles give none, unless they are disrupted with detergents, or the like, when the enzyme becomes accessible. By applying this criterion, incorrect identification can be avoided.

It is now shown that in earlier work the introduction of magnesium ions brought about the formation of right-side-out vesicles in a procedure that would otherwise produce inside-out particles. In this work a series of membrane proteins, which all the available evidence indicates to be at the inner surface, were said to be located on the outer surface. Kant and Steck report that repetition of this work with correctly identified vesicles gave results in agreement with the experiments carried out by other workers using different methods.

position, and leucine and phenylalanine occur only in the second. The sequence as determined fits into the unique indexing system provided by the segment spacings observed in the electron microscope. This method is based on the propensity of the protein, in the right conditions, to form parallel aggregates with all the molecules in register.

The electron microscope reveals after staining a characteristic series of sharp bands, which reflect the discrete distribution of tracts rich in charged residues. The sequence of CB8 can be matched against the pattern of bands of charge in the corresponding segment of the collagen aggregates. A further result that emerges from the new sequence is a reaffirmation of the similarity between  $\alpha_1$  and  $\alpha_2$ —an unsurprising finding, inasmuch as collagens are known in which the three strands are in fact identical. A computer search revealed that the closest homology to  $\alpha_1$ -CB8 in the  $\alpha_2$  chain does indeed occur at the corresponding point in the sequence of the latter.

Kühn and his colleagues, working with calf-skin collagen, have meanwhile sequenced another central part of the  $\alpha_1$  chain, CB3 (Fietzek *et al.*, *FEBS Lett.*, **26**, 74; 1972), consisting of 149 residues. The polar-non-polar alternation, correlating with the electron microscope spacings, is in evidence. Again leucine and phenylalanine, for inscrutable reasons, appear only in the second position of the triplets. The sequence of  $\alpha_1$ -CB3 from rat skin collagen is only partly known, but it seems that it probably differs in no more than about three positions. A comparison of the same fragment derived from the skin and teeth of rats has also been made (Butler, *Biochem. Biophys. Res. Commun.*, **48**, 1540; 1972). Separation and analysis of tryptic peptides show that the two sequences are identical, except that the second is more highly hydroxylated, with one extra hydroxyproline instead of a proline, and substantial, though not complete, modification of two lysines to hydroxylysine. This pattern is thought to apply equally in other parts of the collagen chains.

A large portion of the total sequence, then, is now known, and the pattern is well established. Some problems still surround the unstructured ends of the chain, one of which is the variability between preparations from different sources. Stoltz, Timpl and Kühn (*FEBS Lett.*, **26**, 61; 1972) have now confirmed the surmise that artefacts are generated by endogenous proteases in rat, as in other mammalian collagens. The anomalies are eliminated if the collagen is extracted in denaturing media. Fietzek, Kell and Kühn (*ibid.*, **66**) have also explained the sequences of the first 42 residues of a fragment (CB4) of the  $\alpha_2$  chain from rat and calf

skin, lying near the N-terminus. This shows not only that the corresponding chains of the two species hereabouts are very similar, with only four substitutions, but also allows comparison with the corresponding sequence of the rat  $\alpha_1$  chain, where there are ten substitutions, which is again consistent with the notion of extensive overall similarity.

A very interesting new kind of cross-link has been found in cow-skin collagen by Fairweather, Tanzer and Gallop (*Biochem. Biophys. Res. Commun.*, **48**, 1311; 1972). This is the first known protein cross-link based on histidine, a residue conspicuously scarce in collagen, and is trifunctional. It is presumed to be derived from addition of an imidazole to the known cross-linking species, diaminoformylundecandioic acid. The authors believe that other aldol-imidazole species are also present in the same collagen. Eyre and Glimcher (*Proc. US Nat. Acad. Sci.*, **69**, 2594; 1972) have found a new pattern of reducible cross-links in collagen associated with a genetic human skin disorder. The new components were less pronounced in the bone collagen of the same individuals. As normal cross-links derive from hydroxylysine, it is not surprising that the pathological forms accompany a deficiency in the formation of this amino-acid, which is derived from post-synthetic enzymatic modification of selected lysine residues.

## Pathways to Transformation

IN *Nature New Biology* next week (November 1) Renger reports a series of experiments with temperature sensitive transformed cells which suggest either that DNA and RNA tumour viruses transform cells by independent mechanisms or, if they share a common pathway, that the RNA viruses can specify functions which the small DNA tumour viruses rely on the cell to provide. Recently Renger and Basilico selected mouse 3T3 cells transformed by SV40 which at 32° C behave as typical transformants but which at 39° C have a phenotype typical of untransformed 3T3 cells. Furthermore they showed that the SV40 virus which can be rescued from these cells is wild type, not mutant, and that the cells cannot be re-transformed by wild type SV40 when they are cultivated at 39° C. They concluded, therefore, that the temperature sensitive mutation in these SV3T3 cells is probably in the cell genome rather than in the viral genome.

But if this is the case, what happens when these cells growing at 39° C, at which temperature they behave as untransformed cells, are infected with an RNA tumour virus, mouse sarcoma virus (MSV)? Do the cells retain the untransformed cell phenotype or are they retransformed by the MSV? As

## COMPUTERS

### New Memory Device

from a Correspondent

ALTHOUGH the variable threshold transistor will probably soon be established as the basis of "non-volatile" computer memories, it may eventually face competition. A new and simpler device was described at the recent European Solid State Device Research Conference at Lancaster by Holm-Kennedy and Heald of the University of California.

Designers of small computers, especially of those systems used for control of vehicles in the field, are always on the lookout for a compact, non-volatile memory for their computer. In the field, one cannot spare the time to reload all the instructions into the memory; furthermore, the memory may contain vital collections of new data. Thus the memory cells must be proof against accidental erasure if the power is cut off. Unfortunately, the usual micro-miniature electronic memory circuit, the bistable flip-flop, is volatile. Magnetic memories (cores and tape) are non-volatile, of course, but are often unsuitable because they are bulky and hungry for power.

The first breakthrough was the development of the variable threshold transistor, a metal-insulator-semiconductor (MIS) device which stores infor-

Renger found, MSV can indeed transform these temperature sensitive SV3T3 cells as efficiently as it transforms normal 3T3 cells. Clearly therefore these mutant SV3T3 cells have not completely lost the potential to exhibit the transformed phenotype at 39° C.

There are obviously several ways of explaining these data. First, it may be that DNA and RNA tumour viruses initiate non-overlapping events which have the same end result, namely transformation. Second, one can speculate that RNA and DNA viruses cause transformation by initiating the same train of events, but that transformation by DNA viruses depends on the interaction of a viral with a particular cellular protein whereas transformation by RNA tumour viruses does not because the RNA tumour viruses can specify a protein functionally equivalent to the SV40/cell protein aggregate. Third, Renger and Basilico have not, of course, unambiguously proved that all the SV40 genomes in their temperature sensitive SV3T3 cells are wild type; it is conceivable that the cells contain a temperature sensitive mutant SV40 genome that cannot be rescued and a wild type SV40 genome that can be rescued. Which if any of these hypotheses is correct remains to be seen.



mation in the insulator in the form of trapped charge. The device is simple, very small, and ideally suited for fabrication in large integrated arrays.

The device described by Holm-Kennedy and Heald is based, surprisingly, on a simple semiconductor diode or transistor structure and is the first to employ a phenomenon known as field-induced trapping. The charge state of certain impurities in the lattice of a semiconductor can be changed by applying a strong field which allows carriers to surmount a potential barrier surrounding the impurity that is caused by the existing Coulombic charge on the impurity. The carrier can remain trapped for some time even after the field is removed. In its altered state, the impurity has an increased scattering effect on carriers, so the result is a differential negative mobility effect which may be slow to decay.

Kroemer (*Phys. Rev.*, **109**, 1856; 1958), Ridley and Watkins (*J. Phys. Chem. Solids*, **22**, 155; 1961) and Hilsun (*Proc. Inst. Radio Eng.*, **50**, 185; 1962) proposed that negative mobility characteristics should be used to produce microwave oscillations. In fact, Gunn independently happened upon such oscillations in gallium arsenide in 1963 (*Solid State Commun.*, **1**, 88). The negative mobility in this case is not due to field-induced trapping, but to transferred electron effects. The field-induced trapping effect was demonstrated by Ridley and Pratt in germanium doped with gold, and found to be too slow for microwave purposes (*J. Phys. Chem. Solids*, **26**, 21; 1965).

Holm-Kennedy realized that this slowness of trap population and depopulation could be useful. For certain defects it might be possible to reduce mobility for long periods but in a reversible manner—in other words to control the “dumping” of charge in and out of deep-lying impurity centres in semiconductors—and to use this as the basis of a memory device. Copper in silicon seemed a suitable system and, on the first attempt, Holm-Kennedy and a colleague produced a diode with memory action. The memory effect had a long life and the diode could be left unconnected for weeks in the altered state without significant change. A similar change can also be made in a transistor structure, which increases the range of circuits in which the principle can be made use of. Furthermore, the diode device was multistable (the degree of alteration could be graded), unlike the bistable flip-flop.

As the change of state requires times of the order of milliseconds, the principle may be slow for some memory applications—flip-flops can switch in a few nanoseconds—and it remains to be seen whether the trapping and de-

trapping process can be repeated many millions of times without fatigue, a necessary feature of a computer memory. If no fatigue is evident, this storage principle will constitute a definite improvement over the MIS device.

This latest invention is one of the first uses of a subtle feature of deep-lying defect energy levels in semiconductors. Until now, defects have been regarded as damage, to be minimized or, alternatively, to be used as a blunt instrument to “kill” some unwanted property in a material. This invention points to the possibility of a new generation of devices which use the more subtle properties of defects. Indeed, the point is not being ignored by those who have to do with electronic devices, for, in two conferences this summer, there has been discussion of the use of other types of defect introduction to enhance diffusion and modify carrier lifetimes. The recent increase in basic knowledge about dislocations and the effects of particle bombardment now seems to be percolating through to the benefit of the semiconductor technologist.

#### SYNAPSE

### Resolution of Proteins

from our Neurochemistry Correspondent  
RECENT rapid advances in the isolation of pharmacological receptors have revealed that synaptic events can be analysed at a molecular level, and have suggested that individual protein, or

protein-containing, molecules occupying discrete areas of the surface membrane at the synapse—or elsewhere—may subserve specific functions. The synapse itself is specialized for the transfer of information between nerve cells by chemical transmitters, and receptors, as well as enzymes or carrier sites for the inactivation of transmitters, are clearly part of the molecular equipment developed for carrying out this particular task.

The complex integrative actions of the nervous system, including highly accurate recognition of sites for contact during development, imply, however, further specializations at the synapse, which may require their own particular molecular machinery. As yet little is known about such aspects of synaptic function, but one possible approach to the problem is to make an overall chemical analysis of the synapse with the object of isolating and characterizing any other molecules specific to this region, and hence, possibly, determining their function. A recent paper by McBride and VanTassel (*Brain Res.*, **44**, 177; 1972) describing a protein analysis of components in nerve endings is therefore an encouraging start.

One of the obstacles to performing a meaningful chemical analysis so far has been the difficulty of obtaining a reasonably pure preparation of starting material. The subcellular fractionation methods of Whittaker and others allow the separation from brain homogenates of fractions relatively enriched in nerve endings — “synaptosomes” — using

### Measuring Schwarzschild Coordinates

MANY elegant tests of relativistic properties of massive objects exist only in the mind, as gedanken experiments; equally, all too often practical tests of such parameters involve laborious statistical work or a process of elimination in which all non-relativistic effects are explained away and a tiny residue of otherwise unexplained perturbation is pulled out of the hat as the relativistic effect. Both processes have their faults. In next Monday's *Nature Physical Science* (October 30), however, Banerji describes how an observer in the exterior gravitational field of a star can find whether he is at rest in Schwarzschild coordinates by a technique which is both practicable and elegant.

Banerji does, of course, make some assumptions; the star must be non-rotating, have constant radius, and produce a spectrum including discrete lines. But all these requirements are less drastic than the assumption of weak fields which other students of this problem have made and which Banerji does not. It is reasonable, according to Banerji, to make these assumptions for

the Sun, neglecting rotation and the influence of the planets, so his experiment could be carried out from a test vehicle in the solar system.

An observer on board such a vehicle can determine the local standard of rest first by adjusting his motion so that he sees a constant redshift in the spectra from the star; this ensures no acceleration. To check that there is no constant velocity component producing a Doppler shift in the spectra Banerji suggests studying the orbits of projectiles launched from the test vehicle, and he gives the equations necessary to deduce when the vehicle is indeed at rest relative to the star's coordinate frame. If two observers in two such vehicles carry out the same experiments, it is a simple matter for them to determine, from their measurements at different constant positions relative to that frame, the Schwarzschild mass and the radius of the underlying star. As Banerji says, “a high precision in rocket control and measurement of the redshift must be achieved”; but at least the experiment would work in practice.

sucrose density gradients but it is hard to free these entirely of mitochondria and other membranous material, partly because endings isolated in hypertonic sucrose become shrunken and comparatively resistant to lysis. McBride and VanTassel describe a method for isolating synaptosomes under isotonic conditions using a Ficoll-sucrose gradient. These retain their osmotic properties, and upon lysis release their contents. Thus, by further centrifugation, relatively pure fractions of the various components of the nerve endings—soluble cytoplasm, synaptic vesicles, synaptic plasma membranes and mitochondria from nerve endings—can be obtained.

The proteins of the soluble fraction were precipitated with trichloroacetic acid, and those from membrane fractions were solubilized with SDS detergent; all were subsequently separated by polyacrylamide gel electrophoresis. By comparison with the soluble fraction from cell bodies of neurones and glia, the soluble fraction from terminals contained one unique band and also showed the presence of relatively greater quantities of three bands. Mitochondria from synaptosomes gave a similar profile to that obtained with mitochondria from the cell bodies of neurones and glia, but the gel pattern for synaptic vesicles contained eight bands which were not found in the synaptic plasma membrane fraction. This suggests a difference in chemical composition between the vesicle membrane and the presynaptic membrane, which is difficult to reconcile with the now widely held theory, based on morphological evidence, that synaptic vesicles are formed by invagination and pinching off of the presynaptic membrane. The authors suggest that this may be how vesicles are retrieved after fusing with the presynaptic membrane and releasing their contents.

Previous workers have shown that treatment of synaptic plasma membranes (the membranes derived from synaptosomes after they have released their contents by lysis) with the detergent 'Triton X-100' causes the terminal membrane adjoining the synaptic complex to dissolve, while sparing the apposed pre and postsynaptic membranes forming the junction itself. Under these conditions McBride and VanTassel obtained a fraction which electron microscopy showed to contain paired membrane complexes, but which was contaminated with large amounts of electron-dense amorphous material. The polyacrylamide gel pattern of this fraction included five bands which were not present in that part of the synaptic plasma membrane fraction that is soluble in 'Triton'. This suggests the presence of proteins unique to the membranes of the synapse, although, as

indicated by the authors, further purification is essential before these can be identified. Also preparation of the membrane complexes by dissolution in 'Triton' may not be the method of choice, for the detergent might have some effect on the proteins of the complex—a method involving mechanical disruption of the synaptosome ghosts would perhaps be preferable. Once this has been achieved, the next step will be to attempt to dissociate the pre and postsynaptic membranes and compare their structures.

## STATISTICS

### Fisher Remembered

from a Correspondent

A SYMPOSIUM ON R. A. Fisher's contributions to inference in scientific reasoning, organized by the Fisher Memorial Trust, was held in Gonville and Caius College, Cambridge, between September 18 and 20, 1972. The papers and discussion revolved around the concepts of likelihood and fiducial probability, sufficiency and ancillarity, and randomization in experimental design, introduced by Fisher. Although there was some criticism of attempts to explore these concepts in view of their irrelevance or unimportance in the Bayesian scheme for statistical inference, there was sympathy for the view expressed by Dr A. W. F. Edwards (Gonville and Caius College) in his introduction to the symposium that so many people have such serious doubts about universality of the Bayesian scheme that it is but common prudence to explore the alternatives.

Dr I. Hacking (Peterhouse, Cam-

bridge) and Professor A. Birnbaum (New York and Cambridge Universities) read papers which were of particular interest because the views of both speakers have broadened considerably since their earlier advocacy of the likelihood approach—by Dr Hacking in 1965 and Professor Birnbaum in 1962. Dr Hacking examined a possible logical foundation for likelihood inference in the concept of the explanatory power of a hypothesis. He contrasted explanation with prediction, and concluded that so many extra-likelihood factors enter into inference that a pure likelihood theory is too narrow. Professor Birnbaum subscribed to this general view, but laid greater stress on the importance of an operational ingredient in theories of statistical inference, based on a frequentist view of probability. For this reason he is now more sympathetic to including aspects of the Neyman-Pearson approach in a general theory invoking the confidence concept. Professor Bartlett found the likelihood theory unsatisfactory partly because it does not admit the importance of significance tests and partly because he felt a need to interpret data in the light of a repeated sampling theory. To him, likelihood, sufficiency, and the associated Fisherian concept of information, are important only as adjuncts to classical estimation theory.

The fiducial argument attracted some lively comments. Dr G. Wilkinson (Rothamsted and University of Adelaide) was eloquent in its defence, and exposed the example which led Dr Edwards (in his book *Likelihood*) to reject it: Professor A. T. James (University of Adelaide) showed it at work in two complex multiparametric problems,

### Lambda Proteins *in vitro*

ELUCIDATION of the intricacies of the regulation of the expression of the genes of bacteriophage lambda is one of the most impressive achievements of molecular genetics. But as Gesteland and Kahn point out in next Wednesday's *Nature New Biology* (November 1) surprisingly little is known about the biochemistry of these processes and the proteins involved, with, of course, the exception of the *C1* gene product, the lambda repressor, which has been isolated and characterized by Ptashne. To learn more about the biochemistry of lambda gene expression Gesteland and Kahn have set up a cell-free system which, albeit at a very low efficiency, supports the coupled transcription and translation of lambda DNA.

In their system, which consists in essence of a protein synthesizing system from *Escherichia coli* supplemented with *E. coli* RNA polymerase, added lambda phage DNA is transcribed inefficiently and the resulting RNA is

translated. Furthermore this transcription *in vitro* is specific, for it is repressed by adding lambda DNA repressor.

Using <sup>35</sup>S-methionine as a label for the lambda phage proteins made *in vitro* and DNA from appropriate virulent mutants of lambda, Gesteland and Kahn have established that about 90 per cent of the protein made *in vitro* is read to the right from the so-called *toF* promoter and 10 per cent is read to the left from the *N* promoter. To date attempts to isolate and identify the products of the *N* gene and the *toF* gene amongst the proteins made *in vitro* have not been successful, neither, of course, have repeated efforts to isolate these proteins from infected cells. Gesteland and Kahn are, however, optimistic about their chances of using various lambda and lambda/phage 21 hybrid DNAs to identify at least the product of the *toF* gene and perhaps other early genes, and assay *in vitro* their regulatory activity.



while Mr E. F. Harding (Statistical Laboratory, Cambridge) expounded and criticized Dr Hacking's principle of irrelevance, both in its original form and in a form proposed by Dr Edwards. He then found himself still unable to decide whether one could validly leap the fiducial chasm, to which Professor D. R. Cox (Imperial College, London) answered that he thought one could, but only at the risk of falling flat on one's face on the far side. With added support from Professor G. A. Barnard (University of Essex) and Professor D. A. Sprott (University of Waterloo), it became clear that the fiducial argument is by no means dead.

Technical problems arising in interpreting multiparameter models in the likelihood theory were considered by Professor Sprott and Dr O. Barndorff-Nielsen (University of Aarhus), while Professor Cox and Dr J. A. Nelder (Rothamsted) presented papers on randomization, and statistical inference in practice. On the Tuesday afternoon Dr F. Yates took the chair in an historical session addressed by Sir Harold Jeffreys and Professor E. S. Pearson. Dr O. Irwin added some personal reminiscences which prompted a number of amusing anecdotes from the others. The session was followed by the fifth Fisher Memorial Lecture entitled "Statistical Inference in its Historical Development" by Professor Barnard.

## MOLECULAR BIOLOGY

### Starting DNA Synthesis

from our Molecular Genetics Correspondent

ONE of the puzzles about the control of DNA replication in bacteria is that many cells must have more than one control system. As well as the system which acts on the bacterial chromosome, to ensure that there is one replication for every cell division, some other mechanism exists to control the replication of episomes in those cells that carry these extra circles of DNA in addition to their chromosomes. Episomes and chromosomes each constitute a replicon, in other words.

Although the general operation of the chromosomal control system is well characterized, little is known about the control of episome replication. Each bacterial cell may carry several copies of the F factor, and since this number remains constant in a stable population, they must double during every cell division cycle. In the September issue of the *Proceedings of the National Academy of Sciences* (69, 2706; 1972), Cooper now presents evidence that the manner of this control has a form analogous to that of the bacterial chromosome.

Replication of the bacterial chromosome takes about 40 min at 37° C;

a cell division always follows completion of a round of replication in about 20 min. Because these two times are constant, a round of replication must therefore be initiated 60 min before a division. This means that when bacteria grow rapidly, one round of replication may be initiated before the last has been completed and before the consequent cell division. In other words, the round of replication corresponding to one division cycle may in fact be initiated in the preceding cycle, or even earlier.

Replication of the F factor takes place in a similar manner. The activity of  $\beta$ -galactosidase in cells which have their copies of the lactose operon carried upon an F' factor is proportional to the number of copies of the gene; a doubling in enzyme activity therefore shows that the factor has replicated. By following the activity of  $\beta$ -galactosidase when cells are "shifted up" by changing their growth conditions so that they grow more rapidly, Cooper has found that successive doublings in enzyme activity in induced cultures seem to be related to the divisional cycle in the same manner as chromosome replication—and can therefore be explained by the same idea, that rounds of replication of DNA are initiated when the cell reaches a critical size.

These results conflict with the previous conclusion of Zeuthen and Pato (*Molecular and General Genetics*, 111, 242; 1971) that replication of episomes takes place by a different mechanism. They are therefore comforting, for they restore the idea that—in teleological terms—a successful mechanism is likely

to be used by cells whenever possible in similar circumstances. Titration of cell mass is, accordingly, used to determine initiation of both chromosomal and episomal replication.

An interesting sidelight of these results is that they go some way towards explaining the viability of cells in which the normal replication control has been lost so that bacterial replication comes under control of the F factor. Cells which contain an episome fall into two classes. In one the episome is free in the cell and replicates under independent control from the bacterial chromosome. In the other, the episome is integrated into the bacterial chromosome and is no longer replicated independently—it is repressed and is replicated only as part of the bacterial chromosome itself.

But in a third instance, discovered by Nishimura, Caro, Berg and Hirota (*J. Mol. Biol.*, 55, 441; 1971), the bacterial control system may be inactivated and the cells may survive by placing the bacterial genome under the control of an integrated episome. It is now easy to wonder in retrospect, given Cooper's results, how such cells would achieve coordination of chromosome replication and cell division if the F factor control system were quite different in form from that of the chromosome itself. The general relationship between the control of episomal and chromosomal replication—molecular details of the interaction of regulator proteins with DNA control sites remain to be discovered, of course—now answers this question.

### Left-handed Nucleic Acids

ONE promising way of approaching the problem of helix sense in polynucleotides is by spectroscopic studies of dinucleotides and oligonucleotides. Such oligonucleotides can be called left or right-handed according to the helix sense of the structure which would be generated if the folding in the oligomer were repeated throughout a polymer. The change in optical properties with chain length can be followed, and this allows the spectra of polynucleotide polymers to be catalogued with more certainty.

In next Wednesday's *Nature New Biology* (November 1) Ikehara, Uesugi and Yano report ultraviolet absorption and circular dichroism observations on a dinucleoside phosphate and oligonucleotides in which each nucleoside residue has, in addition to the normal glycosidic linkage, a second covalent bond between the sugar and base. In every nucleoside the base is adenine and the sugar is arabinose, that is the 2' OH is on the opposite side of the sugar ring from its position in the normal ribose sugar. The additional covalent bond is

between C8 of adenine and the oxygen of the ribose 2' OH, and constrains the nucleoside to a particular conformation about the sugar-base link. The analogous compounds in which the 2' O is replaced by a sulphur atom were also studied. From their spectroscopic studies Ikehara *et al.* conclude that the dinucleoside phosphates and oligonucleotides containing these nucleosides have a left-handed helical conformation.

The interest in the work of Ikehara *et al.* goes beyond the handedness of the structure itself. For example they report that the oligomer does not form complexes with poly (U). The development of such studies will be extremely important in investigations of the stereochemical restrictions on double helix formation. The fact that the nucleoside in the polymers studied by Ikehara *et al.*, with its two covalent bonds between sugar and base, is more rigid than a normal nucleotide is also relevant to the mechanism of melting out of nucleic acid secondary structure; some work on this is also reported.

# The Nitrogen Fixation Genes

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**Genetic analysis of nitrogen fixation in free-living bacteria is being used to elucidate processes which in the plant root depend on a complex symbiotic relationship. Increased understanding of the nitrogen fixation mechanism will eventually help to increase the world resources of protein.**

THE chronic world-wide shortage of dietary protein is fundamentally a problem of inadequate reduction of nitrogen to ammonium, the raw material required for building all nitrogenous compounds of the cell. An understanding of the genetic basis of biological nitrogen fixation may pave the way to increased protein production.

The first free-living microorganism able to fix atmospheric nitrogen was isolated from a sample of soil by Winogradsky in 1893<sup>1</sup>. This fermentative bacterium, *Clostridium pasteurianum*, grew luxuriantly at the expense of atmospheric nitrogen gas in a culture medium containing a simple sugar, tap water containing trace minerals, and chalk dust ( $\text{CaCO}_3$ ) as buffer. *C. pasteurianum* produces hardy endospores which remain viable for many years in the soil. A few years earlier, Beijerinck had succeeded in isolating root nodule bacteria from leguminous plants and had shown that they are required for healthy plant development<sup>2</sup>. These classic experiments focused attention on the crucial role played by microbes in the nitrogen cycle and initiated the study of biological nitrogen fixation.

In this century, a variety of new species of nitrogen-fixing organisms have been isolated from many natural habitats<sup>3,4</sup>. These include blue-green algae from hot springs, rice paddies, and even the frigid Antarctic, and bacteria such as *Beijerinckia* from acidic soils and *Azotobacter* from soil and water. In all cases, nitrogen-fixing isolates turned out to be bacteria or blue-green algae; thus we believe that this important process is carried out exclusively by prokaryotic microorganisms<sup>4</sup>.

Nitrogen-fixing microbes have evolved an elaborate enzyme system called nitrogenase, which binds nitrogen gas and reduces it to ammonium<sup>5-10</sup>. For a summary of current concepts of this process, see Fig. 1. Large quantities of cellular energy, in the form of reducing power and ATP, are required<sup>9</sup>. Energy sources used include light energy for blue-

green algae and photosynthetic bacteria, sugars for fermentative bacteria, and various carbon compounds (sugars, organic acids, alcohols) for aerobic bacteria. The root nodule bacteria obtain their energy to drive nitrogen fixation and other cellular processes from products of photosynthesis of the host plant. Various ancillary reactions support nitrogen fixation (Fig. 1).

Nitrogenase is composed of at least two different protein components, one containing molybdenum, iron, and sulphide and the other iron and sulphide; both components are required for activity<sup>5,6</sup>. The metals are thought to make up a portion of the active centre(s) of the enzyme<sup>6</sup>. Functional "hybrid" molecules of nitrogenase have been constructed by mixing components prepared from different species of bacteria<sup>10</sup>, suggesting that there is considerable chemical homology between the nitrogenase enzymes in different organisms. The various partial reactions catalysed by nitrogenase, such as ATP-driven  $\text{H}_2$  evolution and the reduction of the triple-bonded compounds acetylene, cyanide, and azide, are common to all nitrogenases, as is inhibition by carbon monoxide ( $\text{CO}$ )<sup>6</sup>. Nitrogenase activity is routinely assayed by a sensitive gas chromatographic technique which separates and detects ethylene (product) and acetylene (substrate)<sup>11,12</sup>. A single colony of actively fixing bacteria converts enough acetylene to ethylene to give a positive test.

## Nitrogen Fixation Genes of Bacteria

Many microorganisms capable of fixing nitrogen are now known<sup>3,4</sup>. Their apparently similar nitrogenase enzyme system may be coded for by similar structural genes, but the regulatory systems and auxiliary support systems probably differ markedly. Past investigators have observed the workings of the nitrogen fixation genes mainly through studies of spontaneous or induced mutations which alter or even completely block the nitrogen fixation process. More detailed genetic studies have been hampered by the complexity of symbiotic systems and by the absence of methods of genetic transfer in free-living nitrogen fixers.

The variability of the genes governing nitrogen fixation in the root nodule bacteria of leguminous plants (*Rhizobium*) has long been recognized. In a classic monograph on *Rhizobium* published in 1932, Fred, Baldwin and McCoy<sup>13</sup> discuss at length the variable traits of *Rhizobium* regarding patterns of host plant specificity, time of nodulation, abundance of nodulation, and effectiveness of nodule nitrogen fixation. Occasionally these bacteria even lose their capacity to form nodules on host plants. More recently, non-effective strains have been readily produced by various chemical mutagens<sup>14</sup>. The nature of the bacterial genes responsible for functional nodulation involves not only the genes coding for the nitrogenase enzyme system but also genes concerned with the infective process and maintenance of the symbiotic relationship. Plant genes are also involved in symbiotic

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nitrogen fixation<sup>15</sup>; for example, the protein moiety (globin) of leghaemoglobin, a pigment believed to be necessary for symbiotic nitrogen fixation, is coded for by the plant<sup>15</sup>. It is still uncertain whether the nitrogenase genes of root nodules originate from plants or bacteria. The strongest argument favouring a bacterial origin is that most of the nitrogenase is localized within the mature bacteroid<sup>7</sup>, presumably being synthesized there under the direction of the DNA of the bacterium.

symbiotic nitrogen fixation. A revival of interest in this area is to be expected.

There are many species of bacteria and blue-green algae which are able to fix nitrogen in the free-living state. Little is known so far of the genetic basis of nitrogen fixation in most of these organisms. Bacterial mutants which are no longer able to use  $N_2$  gas as a nitrogen source have been made by many workers<sup>22-29</sup>. Karlsson and Barker<sup>22</sup>, the first to isolate nitrogen fixation mutants, described a mutant

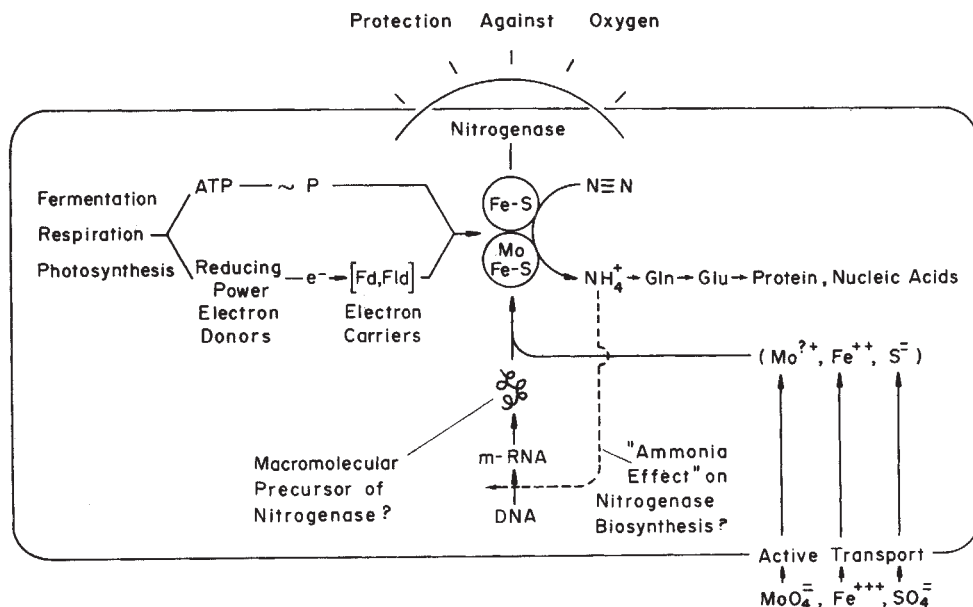


Fig. 1 Summary of current concepts of nitrogen fixation. See text for details. The barrier against inhibitory levels of oxygen is required in aerobic organisms.

There are several reports in the literature of DNA transfer in *Rhizobium*. Balassa<sup>17</sup> has described genetic experiments involving transformation (DNA uptake and recombination) in which as many as 0.05% of the recipient cells were transformed for various nutritional and drug resistance markers. This work was reproduced by Kleczkowska<sup>14</sup>, who was able to obtain ineffective strains of a *Rhizobium* by exposure of cultures to mutagens, virulent phage, or DNA from a spontaneously ineffective variant. Her attempts to construct effective strains of *Rhizobium* from ineffective strains were not successful. Transformation has also been used to transfer plant tumour-inducing ability into *Rhizobium* from the related crown-gall bacterium, *Agrobacterium tumefaciens*<sup>18,19</sup>. A system of conjugation has been reported for one species of *Rhizobium*<sup>20</sup>. Levels of recombination were high, with as many as 10% of the recipient cells receiving DNA from donor cells, but nitrogen fixation markers were not studied. Derivatives of this strain presently maintained apparently do not produce nodules on host plants (Heumann, personal communication); because of keen interest in this organism as a tool for studying the genetic basis of symbiotic nitrogen fixation, a detailed classification of this organism as an authentic strain of *Rhizobium* is anxiously awaited.

Although these two systems of genetic transfer have considerable potential for the study of nitrogen fixation genetics, there are experimental disadvantages to working with *Rhizobium*. The most serious drawback is that the nitrogen fixation genes are apparently expressed only in the symbiotic state, the free-living bacteria being unable to fix nitrogen<sup>7</sup>. The current hypothesis is that the essential genes for nitrogen fixation are activated by compounds present in the micro-environment of the plant nodule. In the past, this has made it necessary to work with whole plants, which is costly in time, effort, and space. The recent development of a *Rhizobium*: plant symbiotic tissue culture system by Holsten and co-workers<sup>21</sup> of the DuPont nitrogen fixation group may prove to be an important technical advance for genetic studies of

of *Azotobacter* which had lost the ability to fix nitrogen when growing on glucose as energy source but could fix nitrogen when glucose was replaced by ethanol. Other substrate-conditional mutants have been isolated from *Azotobacter*<sup>25</sup>, as have mutants which fix in restricted ranges of pH<sup>23</sup> and temperature<sup>29</sup>. Mutant strains which apparently produce defective nitrogenase have been isolated from *Azotobacter* and *Clostridium*<sup>26-28</sup>. Unfortunately, no system of DNA transfer is yet known for these bacteria. The recent report of DNA transformation with blue-green algae<sup>30</sup> may make it possible to study nitrogen fixation genetically in these important free-living nitrogen fixers.

### *Klebsiella pneumoniae* with the *nif* Genes

*Klebsiella pneumoniae*<sup>31</sup> seems a very promising organism for the application of biochemical genetics to the study of nitrogen fixation. It is closely related to *E. coli* and has a similar genetic map<sup>32</sup>. Its ecological role has not yet been fully elucidated, but it has been found in a wide range of habitats, and many strains are able to fix nitrogen under anaerobic conditions, independent of any association with other organisms. Nitrogen-fixing strains of *Klebsiella* have been isolated from leaf nodules of tropical plants<sup>33</sup> and from plant and soil sources<sup>31</sup>. Large numbers occur in the intestines of New Guinea natives whose diet consists mainly of sweet potatoes<sup>34</sup>. Although there is no doubt that nitrogen-fixing strains of *K. pneumoniae* inhabit the human intestinal tract, the ecological significance of *K. pneumoniae* as active nitrogen fixers in the gut and their role in the nitrogen economy of the individual remain unknown.

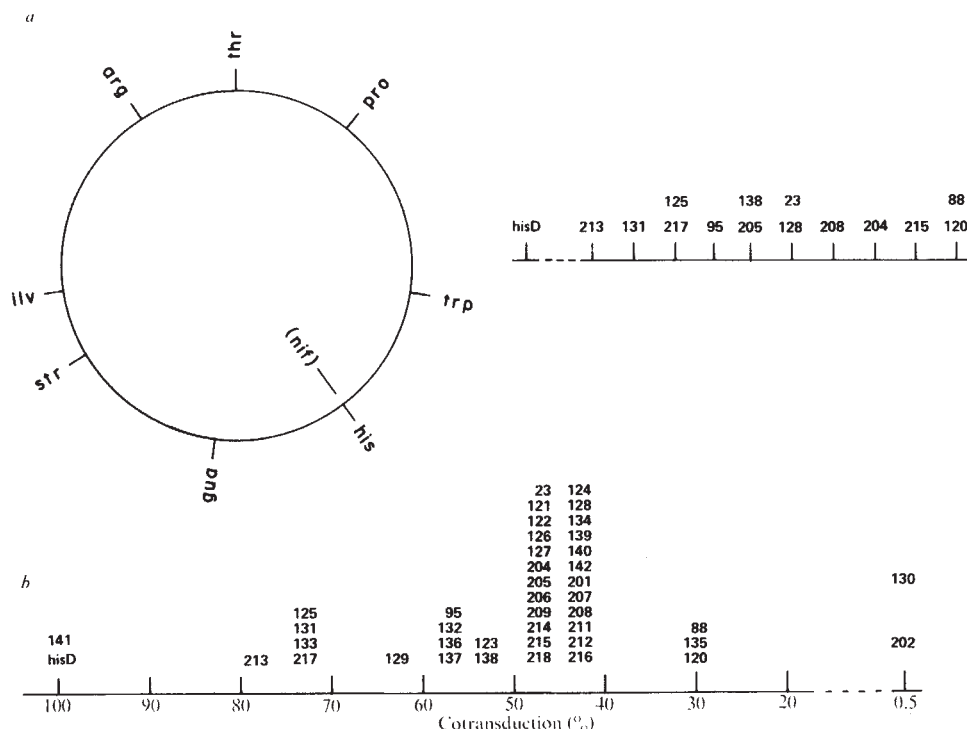
Studies on the genetic basis of nitrogen fixation in *K. pneumoniae* depend upon the availability of a wide range of suitably marked mutant strains and means of genetic transfer between strains. Large numbers of genetically stable mutant strains of *K. pneumoniae* unable to fix nitrogen (nitrogen fixation minus; abbreviated *nif*<sup>-</sup>) are easily

obtained. We have found that two different nitrogen-fixing (*nif*<sup>+</sup>) isolates of *K. pneumoniae* (M5A1 and UW-1, both obtained from P. W. Wilson) are sensitive to the bacteriophage P1 and that P1 behaves as a generalized transducing phage for these strains. Mutants of *K. pneumoniae* M5A1 unable to fix nitrogen are converted to active nitrogen fixers after transduction by P1 carrying DNA from wild type bacteria. The frequency of *nif* gene transfer is in the range of  $10^{-5}$  (i.e., one cell in  $10^5$  being converted to *nif*<sup>+</sup>)<sup>35</sup>. This is about the transduction frequency of other nutritional markers mediated by P1. Transductional analysis (mapping) of the nitrogen fixation genes of this organism is thus possible.

transfer of regions of the bacterial chromosome adjoining the phage integration site (that is, specialized transduction)<sup>39</sup>. Phage 424 is modified and restricted (see Arber and Linn<sup>40</sup> for a review of restriction and modification) by these two strains of *K. pneumoniae*. For example, lysates prepared on strain M5A1 and tested for infectivity on strain UW-1 and on *E. coli* K-12 formed plaques at efficiencies of about  $10^{-5}$  and  $10^{-3}$ , respectively. This must be taken into account in future studies of interstrain transduction mediated by this phage.

Conjugation was first reported in *K. pneumoniae* by Matsumoto and Tazaki<sup>32</sup>, who screened a number of isolates for transfer of nutritional markers. A suitable donor and

**Fig. 2** *a*, Genetic linkage map of *Klebsiella pneumoniae*, modified from Matsumoto and Tazaki<sup>32</sup>. *b*, Summary of cotransduction frequencies of *nif* genes with *his*. Percentages below the line refer to the cotransduction frequencies of the various *nif* mutations with *hisD*; the various mutants studied are listed above the line. Dashed lines refer to unlinked genes. See ref. 35 for experimental details. *c*, Ordering of several *nif* loci near *his* using three-factor transductional crosses; distances between loci are not drawn to scale. The mutational order is determined by comparing the results from reciprocal crosses in which each *nif*<sup>-</sup> mutation is used as donor and recipient.



As mentioned above, we have found two natural isolates of *K. pneumoniae* which are P1 sensitive. The other strains which we have tested (twenty-five isolates) are P1 resistant. These strains, which have varying abilities to fix nitrogen, cannot be studied genetically using P1 transduction. The production of P1-sensitive strains from resistant strains of coliform bacteria has been reported by Okada and Watanabe<sup>36</sup>. We have found that it is possible to select P1-sensitive mutants of *K. pneumoniae* from resistant strains after mutagenesis with nitrosoguanidine. The frequency of conversion from P1 resistance to P1 sensitivity was relatively high, being about 0.1% and 1.0% with two different strains of *K. pneumoniae* (CDC No. 1702-49 and No. 2844-64; see ref. 31). This method of increasing the host range of the generalized transducing phage P1 makes it possible to investigate other strains of *K. pneumoniae* and perhaps other coliform bacteria genetically. It should also be possible to determine the defects in some of the natural isolates and correct them by transferring in the nitrogen fixation genes from other strains.

We have recently observed that the nitrogen-fixing strains M5A1 and UW-1 are also sensitive to the lambdoid phage 424, which is known to integrate into the *E. coli* chromosome near the histidine (*his*) operon<sup>37</sup>, a cluster of genes required for histidine biosynthesis<sup>38</sup>. Other lambdoid phages of *E. coli* (for example,  $\lambda$  and  $\phi$ -80) are known to mediate the

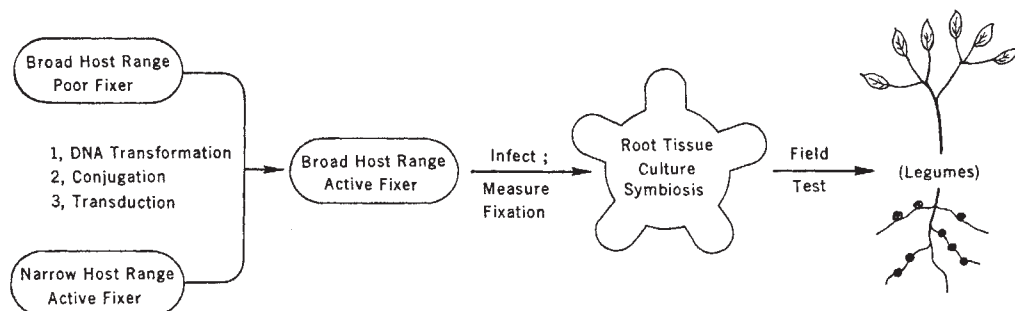
recipient pair were found among natural isolates from hospital patients and used to establish a genetic linkage map for *K. pneumoniae* (Fig. 2a). Approximately fifty markers have been located so far; but these strains do not fix nitrogen. Dixon and Postgate<sup>41,42</sup> have prepared a donor strain capable of transferring the *nif* genes by introducing a derepressed R-factor into *K. pneumoniae* M5A1. *K. pneumoniae* cells harbouring the derepressed R-factor transferred the R-factor with great facility and occasionally donated chromosomal DNA including genes for nitrogen fixation to *K. pneumoniae* M5A1 and *E. coli*. This conjugation system should facilitate genetic studies in this field.

### Location of *nif* Operon(s)

Nitrogen-fixing organisms contain DNA which codes for the structural and regulatory genes governing nitrogenase and other essential functions of nitrogen fixation. These genes might be located in different regions of the chromosome or might be organized as tightly clustered and functionally related groups of genes ("*nif* operons"). Recent genetic and biochemical evidence suggests that *K. pneumoniae* has at least one *nif* operon located near the *his* operon.

Transductional analysis has revealed that most *nif* genes are clustered near the *his* operon in *K. pneumoniae*. As summarized in Fig. 2b, the frequency of P1 cotransduction





**Fig. 3** Hypothetical scheme for genetic hybridization and testing of improved strains of root nodule bacteria (*Rhizobium*) of leguminous plants. The scheme calls for application of techniques of molecular biology and modern plant breeding.

of most *nif* markers with *his* ranges from 78% for *nif-213* to 30% for *nif-120* and *nif-135*. A. L. Taylor has kindly analysed these data and estimates this region to be fifteen to twenty average gene lengths in size. This would be more than sufficient DNA to code for all proteins known to be involved in nitrogen fixation. Of the forty-two *nif*<sup>-</sup> strains listed in Fig. 2b, thirty-nine are genetically linked with *his*. We have found very few *nif* mutants with lesions in other regions of the chromosome. Two mutations, *nif-130* and *nif-202*, seem to be unlinked with *his*, for no *nif*<sup>+</sup> colonies were found among the *his*<sup>+</sup> transductants. They are both phenotypically similar to the *nif* mutants which map near *his* and are not linked to each other. They presumably represent new *nif* genes located in different regions of the *K. pneumoniae* chromosome.

An unusual class of *nif*<sup>-</sup> mutants was isolated by selecting for simultaneous *his*<sup>-</sup> *nif*<sup>-</sup> double auxotrophs. As shown in Fig. 2b, *nif-141*, a typical strain in this group, shows 100% linkage between *his* and *nif* markers. A single base mutation is indicated since prototrophic revertants are readily obtained. The nature of this lesion blocking both pathways is unknown but may be regulatory in nature.

The relative order of several *nif* loci located near *his* has been determined through the use of three-factor transductional crosses (Fig. 2c). The orientation of these *nif* genes and the *his* genes relative to other markers on the chromosome has not been determined. Few convenient markers are known in this region of the chromosome. We have evidence that *K. pneumoniae* has a P2 prophage site in this region. We have found that *K. pneumoniae* M5A1 is sensitive to the phage P2 and have isolated eductants, mutants isolated from P2 lysogens which have large deletions extending through the *his* operon and adjacent genes<sup>43</sup>. These eductants lack the genes of the *his* operon and the enzyme gluconate-6-phosphate dehydrogenase (*gnd*), are unable to fix nitrogen, and produce no detectable nitrogenase. The eduction process in *E. coli* involves deletion of a P2 prophage carried in location H, a site found in *E. coli* K strains which maps close to the *his* operon<sup>44</sup>. Thus, *K. pneumoniae* M5A1 has a P2 prophage site similar to location H, and mapping of this prophage site may determine the relative order of *his* and *nif* on the chromosome.

The conjugational transfer of the *his*-linked *nif* region from *K. pneumoniae* to *E. coli* achieved by Dixon and Postgate<sup>45</sup> provides additional evidence that the *nif* gene cluster near *his* may be a *nif* operon. *E. coli* does not naturally fix nitrogen, but they were able to obtain *E. coli* which had received *his* genes from their *K. pneumoniae* donor strain and also had a fully functional nitrogenase system. The simplest interpretation of this startling result is that structural and regulatory genes for nitrogenase are located near *his*.

Biochemical evidence also supports the concept of a major nitrogenase operon(s) near *his*. An overwhelming majority of the mutants isolated from *Klebsiella* lack an active nitrogenase system. For example, of 138 *nif*<sup>-</sup> mutants that were assayed for whole cell nitrogenase activity, about two-thirds reduced acetylene at a rate less than 0.5% that of the wild type strain. Twenty-five mutants gave rates of acetylene reduction from 0.5–1% that of wild type, while five strains

were 1–2% as active; seventeen strains were "leaky" in their growth on N<sub>2</sub> and had acetylene reduction capacity from 2–38% that of wild type. Most of these mutants were found to map near *his*. It seems clear that most *nif*<sup>-</sup> strains which we have isolated are blocked at the level of nitrogenase itself.

At least two genes, corresponding to the Mo-Fe-protein and the Fe-protein, must code for nitrogenase. Mutations in either gene would completely destroy nitrogenase activity since neither component can function catalytically without the other. *In vitro* complementation between mutants which map in the *nif* region near *his* indicates that structural genes for nitrogenase are located in this region (Streicher and Valentine, in preparation).

## Regulation of Nitrogen Fixation

Ammonium ion, the product of the nitrogen fixation reaction, controls the synthesis of nitrogenase in *Klebsiella*; the addition of ammonium to a culture growing on nitrogen gas represses synthesis of the nitrogenase system<sup>3,9,46</sup>. The molecular basis of the repression of nitrogenase by NH<sub>4</sub><sup>+</sup> is not yet understood. It is thought that NH<sub>4</sub><sup>+</sup> exerts its control at the level of gene expression. Ammonium apparently does not inhibit nitrogenase activity by direct feedback inhibition of catalytic activity<sup>9</sup>. In certain organisms, ammonium is known to control not only the production of the nitrogenase enzyme system but also the synthesis of specialized cell structures thought to be essential for nitrogen fixation in these species. For example, available ammonium represses the formation of algal heterocysts, specialized cells formed by many strains of blue-green algae when fixing nitrogen<sup>3,46</sup>.

In *Klebsiella*, other enzymes as well as nitrogenase are repressed by NH<sub>4</sub><sup>+</sup><sup>47</sup>. We refer to this phenomenon as the "ammonium effect" because of its superficial similarity to the "glucose effect", a general regulatory system governing energy metabolism in bacteria. The repression of the histidine utilization (*hut*) genes by NH<sub>4</sub><sup>+</sup> has been studied in detail by Magasanik and coworkers. Recently, Prival and Magasanik<sup>47</sup> reported that the regulation of histidase, the first enzyme involved in histidine utilization, by ammonium does not involve cyclic AMP. This work was carried out with mutant strains of *K. aerogenes* unable to synthesize cyclic AMP. We have constructed similar cyclic AMP deficient strains of *K. pneumoniae* M5A1 to study the role of cyclic AMP in the regulation of nitrogenase. In agreement with the results of Prival and Magasanik with histidase, we found no effect of cyclic AMP on nitrogenase synthesis; that is, the induction of nitrogenase (acetylene assay) takes place in the mutants as in wild type and is not strongly influenced by the addition of exogenous cyclic AMP (5 × 10<sup>-3</sup> M).

Regulatory mutants governing nitrogenase have not yet been clearly defined but are of considerable interest. Several mutants we have isolated may have altered regulatory properties. The lesion which maps in the histidine region (*nif-141*) is a pleiotropic mutation blocking both histidine production and nitrogen fixation. Interestingly, the nitrogen fixation and *his* defects were overcome by transfer of an F'*his* episome derived from *E. coli* into the mutant; this may be indicative

of a positive control mechanism which activates both pathways.

We have investigated the idea that mutants blocked at the level of the "ammonium effect" might be isolated. Mutants unable to utilize any of a series of nitrogenous compounds are readily isolated<sup>49</sup>. These mutants are also unable to multiply with  $N_2$  as sole nitrogen source although nitrogenase, as determined by the acetylene assay, is induced. The biochemical deficiency of these strains is the lack of glutamate synthetase, the second enzyme in the pathway of ammonium assimilation<sup>49</sup>. The mutants synthesize considerably less nitrogenase than wild type when induced in the presence of  $N_2$ . The low levels of nitrogenase observed during growth on  $N_2$  may be the result of repression by  $NH_4^+$ , glutamine, or an unknown metabolite which might accumulate in the mutants because of defects in the pathway of ammonia assimilation. Induction of nitrogenase in the absence of  $N_2$  (aspartate containing media) results in comparable enzyme levels with wild type. The genetic lesions do not appear to be linked with *his* or any of the *nif* loci.

It is interesting to speculate that the diffusible substance(s) proposed by Prival and Magasanik<sup>47</sup> to mediate the "ammonia effect" includes a protein which binds to the promoter region of the *nif* operon and activates the transcription of messenger RNA for nitrogenase biosynthesis.  $NH_4^+$  or one of its metabolites might repress the synthesis of nitrogenase by blocking or altering the DNA binding site of the activator protein. Exogenous ammonium presumably enters the cell by way of a specific active transport (permeation) system; cellular ammonium is assimilated yielding amino-acids, nucleotides, and finally proteins and nucleic acids, any of which are candidates for mediating ammonium repression. The first enzyme of the pathway of ammonium assimilation during nitrogen fixation, glutamine synthetase, has a number of striking features which have focused attention on this enzyme (and its product, glutamine) as possibly being involved in ammonium repression. This enzyme shows an amazing responsiveness to ammonium levels in the cellular environment<sup>49,50</sup>. The bio-synthetically active, nonadenylylated form, is present in the nitrogen-fixing or ammonium-limiting cell and is rapidly converted to the inactive, adenylylated form by the addition of ammonium ion. Changes in the pattern of glutamine synthesis and ammonium assimilation brought about by the ammonium regulated covalent modification of glutamine synthetase may be linked to ammonium repression of nitrogenase synthesis.

Dixon and Postgate<sup>48</sup> mention that the *nif* genes of *E. coli*/*Klebsiella* hybrid cells are still repressed by  $NH_4^+$ . The logical interpretation of this experiment is that the ammonium effect is operative and that regulatory sites on the DNA contributed by *K. pneumoniae* are involved. The important implication of this experiment is that regulatory elements located near the *his*-linked *nif* genes function during  $NH_4^+$  repression.

## Practical Applications

The work with *K. pneumoniae* has shown that there are more genes governing the regulation and synthesis of the nitrogen fixation system than was expected. Similarly, the genome of agronomically important species such as the root nodule bacteria may contribute a larger genetic share to the symbiotic nitrogen fixation than currently appreciated. In the future, bacterial genes determining host specificity, survival in different soil conditions, levels of nitrogenase, selective patterns of synthesis of organic nitrogenous compounds, and various other physiological parameters might be manipulated by genetic hybridization, as indicated in Fig. 3, leading to improvement of field crops. In proceeding toward this practical goal, it is essential to obtain more basic information about the nitrogen fixation genes and their control. The studies with *K. pneumoniae* have opened the door to analysis of this important system using the penetrating tools of molecular biology.

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This article is dedicated to C. B. Van Niel for his contributions to microbiology and man.

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# Chemotaxis in *Escherichia coli* analysed by Three-dimensional Tracking

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**Chemotaxis toward amino-acids results from the suppression of directional changes which occur spontaneously in isotropic solutions.**

If a capillary tube containing an attractant is inserted into a suspension of motile bacteria, the bacteria accumulate near the mouth where the concentration of attractant is relatively high. Pfeffer<sup>1</sup> introduced this technique as an assay for chemotaxis in 1884. In 1901, Rothert<sup>2</sup> and Jennings and Crosby<sup>3</sup> noted that bacteria often swam past the capillary but returned after failing to enter regions of much lower concentration. It is now generally thought that chemotactic bacteria actively avoid regions of lower concentration by backing up or by choosing new directions at random<sup>4,5</sup>. This is not obvious in *Escherichia coli*, since these bacteria repeatedly change their directions even in the absence of an applied stimulus.

To study this motion in detail, we built a microscope which automatically follows individual cells<sup>6</sup>. We have used this on mutants of *E. coli* K12<sup>7</sup>: a wild type<sup>8</sup>, a nonchemotactic mutant<sup>8</sup>, an uncoordinated mutant<sup>9</sup>, a mutant defective in taxis toward serine<sup>10,11</sup> and a mutant defective in taxis toward aspartate<sup>11</sup>. The results which we describe here demonstrate that the response to serine and aspartate at concentrations of order  $10^{-5}$  M is not an avoidance response; when cells swim down gradients of these amino-acids their motion is indistinguishable from that in isotropic solutions; when they swim up the gradients they change direction less frequently.

In another communication<sup>12</sup> we discuss a solution to the diffusion equation which allows us to compute the concentration of attractant outside the mouth of a capillary, and, thus, to follow cells in defined gradients.

## Motion in Isotropic Solutions

The motion appears as an alternating sequence of intervals during which changes in direction are gradual or abrupt—we call these “runs” and “twiddles”, respectively. Genetic and environmental differences in behaviour are associated chiefly with the lengths of runs. Fig. 1 illustrates this for the wild type and a nonchemotactic mutant. A number of results of a quantitative run-twiddle analysis are given in Table 1.

Runs are long in *cheC* 497, short in *unc* 602, and of intermediate length in AW405 (Table 1). Mutants able to respond to a restricted set of attractants swim much like the wild type; the motion of the serine-blind mutant AW518 is essentially identical to that of AW405; the aspartate-blind mutant AW539 has somewhat shorter twiddles and somewhat longer runs

( $0.11 \pm 0.18$  s and  $1.3 \pm 2.1$  s, respectively). The speed is nearly uniform during runs, but the bacteria slow down or stop on twiddling (Fig. 2). The mean change in direction from the end of one run to the beginning of the next is less than  $90^\circ$  (Table 1). If the bacteria chose a new direction at random, the probability of an angle change between  $\theta$  and  $\theta + d\theta$  would be  $1/2 \sin \theta d\theta$ , the mean value of  $\theta$  would be  $90^\circ$ , and the standard deviation would be  $39.2^\circ$ . The distribution observed, however, is skewed toward small angles (Fig. 3). If changes in direction were random, the skew would be toward large angles because the digital

**Table 1** Run-twiddle Analysis of Mutants Swimming in a Homogeneous, Isotropic Medium

| Strain Type                                                  | AW405 Wild type | <i>Unc</i> 602 Uncoordinated | <i>CheC</i> 497 Nonchemotactic |
|--------------------------------------------------------------|-----------------|------------------------------|--------------------------------|
| Number of bacteria tracked                                   | 35*             | 10                           | 14                             |
| Total tracking time (min)                                    | 20              | 3.0                          | 2.7                            |
| Mean speed ( $\mu\text{m/s}$ )†                              | $14.2 \pm 3.4$  | $14.4 \pm 3.9$               | $20.0 \pm 4.9$                 |
| Mean twiddle length (s)‡                                     | $0.14 \pm 0.19$ | $0.14 \pm 0.24$              | $0.10 \pm 0.13$                |
| Mean run length (s)                                          | $0.86 \pm 1.18$ | $0.42 \pm 0.27$              | $6.3 \pm 5.2$                  |
| Mean change in direction from run to run ( $^\circ$ )        | $68 \pm 36$     | $74 \pm 33$                  | $33 \pm 15$                    |
| Mean change in direction during runs ( $^\circ$ )            | $23 \pm 23$     | $18 \pm 23$                  | $35 \pm 22$                    |
| Mean angular speed while twiddling ( $^\circ/\text{word}$ )§ | $56 \pm 29$     | $54 \pm 27$                  | $41 \pm 32$                    |
| Mean angular speed while running ( $^\circ/\text{word}$ )§   | $14 \pm 9$      | $19 \pm 9$                   | $9 \pm 6$                      |

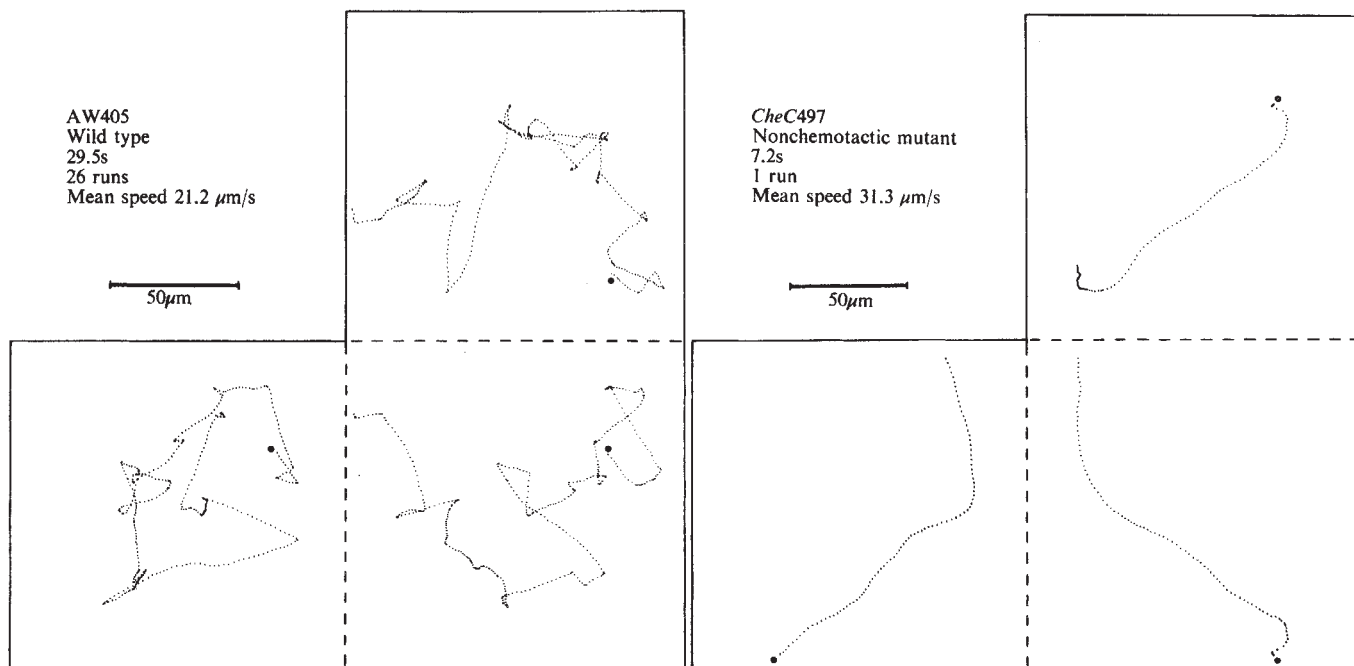
Data points (words) were generated at the rate of 12.6 per second. The beginning of a run was scored if the angular speed § was less than  $35^\circ/\text{word}$  for three successive words. The end of a run was scored if the angular speed was greater than  $35^\circ/\text{word}$  for two successive words or if it was greater than  $35^\circ/\text{word}$  for one word, provided, in the latter case, that the change in the average direction between successive pairs of words was also greater than  $35^\circ$ . The angular speed is sensitive to short term fluctuations in the data. These depend on the ways in which the bacteria wobble and on the time constants (0.08 s) of the circuits which precede the analogue-to-digital converter. The time constants, the recording rate and the value  $35^\circ/\text{word}$  were chosen empirically by comparing results of digital analyses with plots of the kind shown in Fig. 1.

\* Experiments done with three different cultures.

† The values are the means  $\pm$  one standard deviation. In the calculation of the mean speed the mean for each bacterium is weighted equally, and the standard deviation is the standard deviation in the mean.

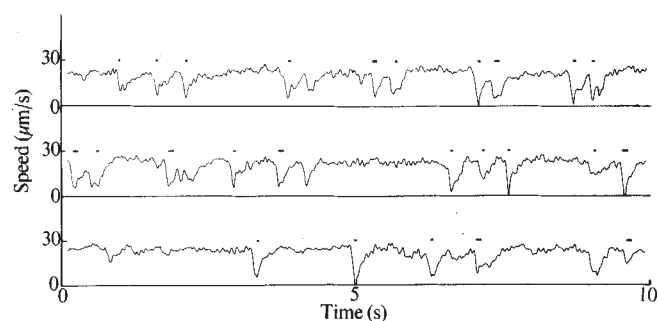
‡ In this and in subsequent entries in the table each twiddle or run is weighted equally; the standard deviations are of the same order of magnitude as those found with a single bacterium.

§ The angular speed is the change in the direction of motion from one word (data point) to the next.



**Fig. 1** Digital plots of the displacement of a wild type bacterium, AW405, and a generally nonchemotactic mutant, *cheC* 497, at the rate of 12.6 words (data points) per second. Tracking began at the points indicated by the large dots. The plots are planar projections of three-dimensional paths. If the left and upper panels of each figure are folded out of the page along the dashed lines, the projections appear in proper orientation on three adjacent faces of a cube. The cultures were grown in a minimal salts medium on glycerol, threonine, leucine, and histidine, as described by Hazelbauer *et al.*<sup>10</sup>. They were washed twice at 4° C with a solution containing 10<sup>-2</sup> M sodium phosphate (pH 7.0), 10<sup>-4</sup> M EDTA (ethylenediamine tetraacetate) and 10<sup>-3</sup> M magnesium sulphate and diluted at room temperature to an optical density of 0.01 (590 nm) in a solution containing 10<sup>-2</sup> M sodium phosphate (pH 7.0), 10<sup>-4</sup> M EDTA, and 0.18% (w/v) hydroxypropyl methylcellulose (Dow Methocel 90 HG). They were tracked as such at 32.0° (viscosity 2.7 cp) in a tantalum and glass chamber 2 mm in diameter and 2 mm high.

analysis ignores the smallest changes (Table 1, legend). Changes in direction also occur during runs (Table 1). The drift is about what one would expect from rotational diffusion: the root-mean-square angular deviation of a 2 μm diameter sphere occurring in  $t$  sec in a medium of viscosity 2.7 cp at 32° C is  $29\sqrt{t}$  degrees.

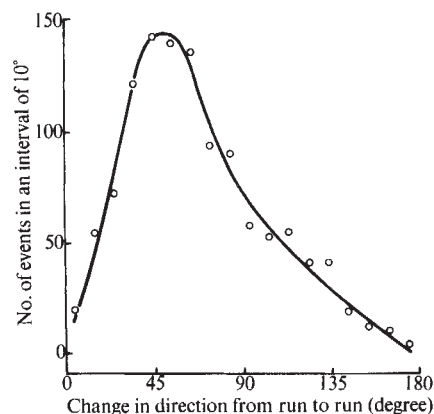


**Fig. 2** The speed of the wild type bacterium of Fig. 1 displayed by an analogue monitor. The recording has been divided into three parts, each 9.8 s long; the figure should be read from left to right and top down. Twiddles occurred during the intervals indicated by the bars. Note the consequent changes in speed. The longest run can be seen at the left end of the bottom trace. It appears in the upper panel of Fig. 1 angling downwards and slightly to the left, five runs from the end of the track. It is 45 words or 3.57 s long.

The shortest twiddles and the shortest runs are the most probable (Fig. 4). The distribution of twiddle lengths is exponential (Fig. 4a). The distribution of run lengths is exponential for *unc* 602 (not shown) but only approximately so for AW405 (Fig. 4b). If for AW405 one allows for variations in mean run length for different bacteria, the curvature in the semi-log plot of the aggregate run-length data vanishes (Fig. 4c).

From calculations of autocorrelation functions of sequences of twiddles and of sequences of runs we conclude that twiddles and runs of different length occur at random. The statistics are Poisson; for a given organism in a given isotropic environment the probability per unit time of the termination of a twiddle or the termination of a run is a constant.

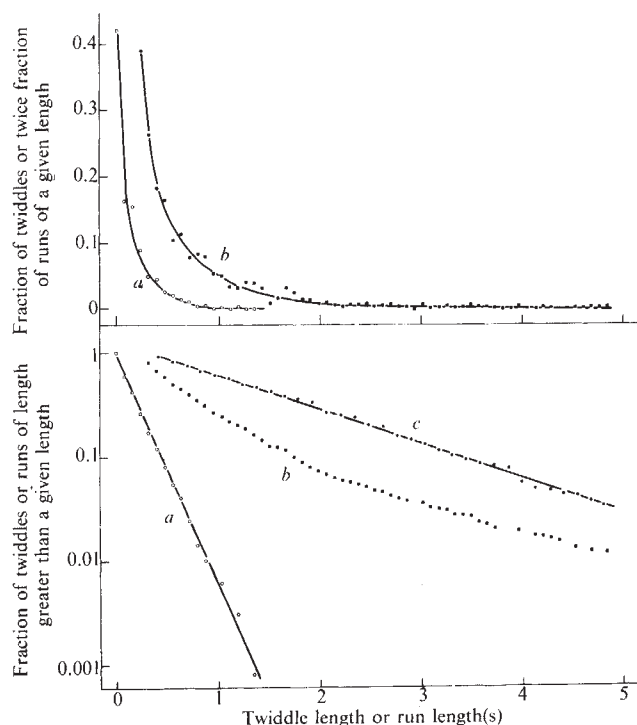
The wild type is known to have chemoreceptors for serine, for aspartate and for a number of sugars<sup>7</sup>. If serine is added to suspensions of AW405 (no gradients), the run-length distributions remain exponential but shift dramatically toward longer runs (Fig. 5); the twiddles are suppressed. Calculations of the autocorrelation functions indicate that runs of



**Fig. 3** Distribution of changes in direction from the end of one run to the beginning of the next for the wild type bacteria of Table 1. The distribution was constructed from 1,166 events by summing the numbers falling in successive 10° intervals. If the analysis is confined to the shortest twiddles, the distribution is skewed even farther toward small angles (mean and standard deviation  $62 \pm 26^\circ$ ).



different length still occur at random. The shift does not occur with aspartate (Fig. 5), even though the chemotactic responses to aspartate and serine are nearly the same<sup>10,11</sup>. The shift due to serine involves the serine chemoreceptor; it does not occur in the serine-blind mutant AW518. The shift is not a metabolic effect, since it can be generated by a non-metabolite sensed by the serine chemoreceptor (experiment done with  $\alpha$ -amino-isobutyric acid<sup>11</sup> and the aspartate-blind mutant AW539, which also shows the shift with serine). It does not occur in the uncoordinated mutant *unc 602*. Adler<sup>7</sup> notes that "serine slightly inhibits chemotaxis toward all other attractants, and this inhibition remains unexplained". If chemotaxis results from the suppression of twiddles (see below), serine should inhibit chemotaxis generally, provided that the mutants tested have a functional serine receptor.



**Fig. 4** Top: the fractional number of twiddles (*a*) or runs (*b*) of different lengths for the wild type bacteria of Table 1. There were 1,201 twiddles and runs. All the twiddles are plotted, but runs longer than 5 s are not (lengths 5.1, 5.4, 6.0, 6.4, 7.0, 7.1, 7.1, 7.2, 7.9, 7.9, 12.9 and 24.2 s). Bottom: the same data plotted as the logarithm of the fractional number of twiddles (*a*) or runs (*b*) of length greater than a given length<sup>13</sup>. Curve *c* was obtained by scaling the run lengths of each bacterium so that its mean run length was equal to the ensemble mean.

Serine has other effects on the motion of the wild type which are less dramatic but which have a similar concentration dependence. The mean speed increases by about 40%; the mean twiddle length, the mean change in direction from run to run, and the mean angular speed while running all decrease by about 40%. With aspartate there is a slight increase in speed, but the other changes are not significant.

## Motion in Gradients

Gradients were generated by diffusion of attractants from capillary tubes of the kind used by Adler<sup>7</sup>, which we inserted through a flat side wall of the tracking chamber. In preliminary experiments we found the response (the number of bacteria entering a capillary in 1 h) to be negligible when bacteria were used at an optical density of 0.1 (590 nm; about 10<sup>8</sup> bacteria/ml.); the clouds of bacteria which accumulated near

the mouths of the capillaries sank. At optical densities of order 0.01 the response to an attractant was proportional to the optical density, and the dependence on the concentration of the attractant was similar to that described by Adler<sup>7,11</sup> (experiments at 32° with serine, aspartate,  $\alpha$ -amino-isobutyric acid and  $\alpha$ -methyl-DL-aspartate, all in tracking medium).

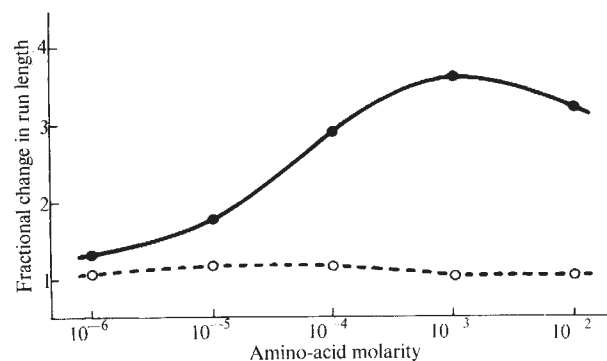
**Table 2** Run-twiddle Analysis of the Wild Type Swimming in Gradients

| Attractant                                                    | Serine      |             | Aspartate   |             |
|---------------------------------------------------------------|-------------|-------------|-------------|-------------|
|                                                               | Control     | Gradient    | Control     | Gradient    |
| Number of bacteria tracked                                    | 11          | 34          | 23          | 24          |
| Total tracking time (min)                                     | 7.1         | 14.8        | 11.1        | 11.0        |
| Mean run length (s)                                           | 0.83 ± 0.88 | 1.67 ± 2.56 | 0.83 ± 0.90 | 0.90 ± 1.56 |
| Mean concentration of attractant (μM)                         | 0           | 9.5 ± 2.7   | 10          | 8.4 ± 2.0   |
| Mean distance from mouth of capillary (μM)                    | —           | 577 ± 112   | —           | 644 ± 88    |
| Mean value of $(\partial C/\partial r)/C$ (mm <sup>-1</sup> ) | —           | 2.5 ± 0.4   | —           | 2.4 ± 0.4   |

The cells were prepared as described in the legend of Fig. 1, except that the suspension used for the aspartate control also contained 10<sup>-5</sup> M aspartate. Capillaries were used in the gradient experiments but not in the controls. For each data point we computed the distance from the mouth of the capillary to the bacterium being tracked (*r*), the angle between the direction of motion of the bacterium and the gradient (the "inclination", 0° for motion radially down the gradient), the concentration of the attractant at the bacterium (*C*, as defined by equation (4), ref. 12, with  $r_c = 0.01$  cm,  $C_0 = 2.0 \times 10^{-3}$  M,  $D_{\text{serine}} = 1.0 \times 10^{-5}$  cm<sup>2</sup>/s and  $D_{\text{aspartate}} = 0.89 \times 10^{-5}$  cm<sup>2</sup>/s), the steepness of the gradient at the bacterium ( $\partial C/\partial r$ ), the time rate of change of the concentration at the bacterium ( $dC/dt$ ), and the logarithmic derivatives  $(\partial C/\partial r)/C$  and  $(dC/dt)/C$ .

Mean speeds, twiddle lengths, changes in direction and angular speeds are not shown; the values are essentially identical to those for AW405 (Table 1).

Results of the run-twiddle analysis for the gradient experiments are given in Tables 2 and 3. Runs are longer in the gradients (Table 2) than we would expect from the concentration dependence (Fig. 5). This is true for runs which move the bacteria up the gradient but not for runs which move them down the gradient (Table 3). The differences in the up-gradient and down-gradient data are dramatic when the run-length distributions are examined (Fig. 6). For serine, the distri-



**Fig. 5** Changes in mean run length caused by serine (●) or aspartate (○). The bacteria (AW405) were diluted in the tracking medium (Fig. 1, legend) to an absorbance of 0.02 (590 nm). Aliquots of this suspension were mixed with equal volumes of tracking medium containing L-serine or L-aspartate (Calbiochem A grade). Controls were made by omitting the amino-acids. The ratios of the mean run lengths observed in the presence and in the absence of the amino-acid are plotted as a function of concentration.

bution of runs down the gradient (Fig. 6b) is similar to the distribution in a 9  $\mu\text{M}$  isotropic solution; for aspartate, it is indistinguishable from the control. From the data of Fig. 6 and from calculations of autocorrelation functions of sequences of runs (not separated into subsets), we conclude that the statistics are still Poisson. When a bacterium moves up the gradient the probability per unit time of the termination of a run decreases; when it moves down the gradient the probability reverts to the value appropriate to an isotropic solution of similar concentration. At the concentrations we have studied, the stimulus is sensed and acted on only when the bacterium swims up the gradient.

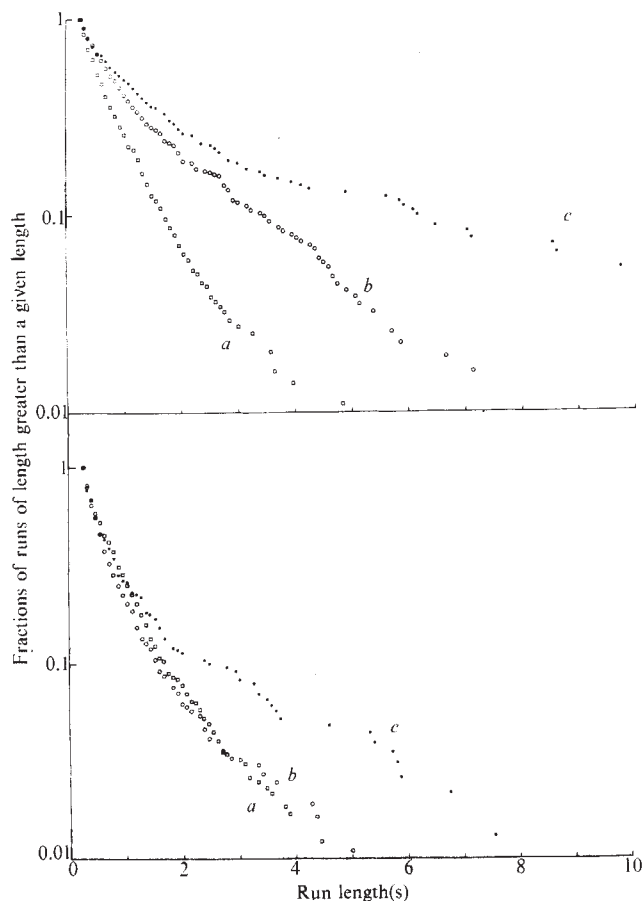


Fig. 6 The data from the serine (top) and the aspartate (bottom) experiments (Tables 2 and 3) plotted as the logarithm of the fractional number of runs of length greater than a given length. *a*, Runs in the control experiment; *b*, runs down the gradient; *c*, runs up the gradient.

Further proof of the assertion that motion away from the capillary is not sensed can be obtained from computation by linear regression<sup>14</sup> of the correlation between the length of a run and the mean value over the run of  $dC/dt$ ,  $(dC/dt)/C$ ,  $-\partial C/\partial r$ , or  $-(\partial C/\partial r)/C$ . These correlations are all positive (correlation coefficients of order  $0.12 \pm 0.02$ ). If the analysis is confined to runs which move the bacteria up the gradient, the correlation coefficients are larger (of order  $0.19 \pm 0.03$ ); if it is confined to runs which move the bacteria down the gradient, the coefficients are statistically insignificant ( $-0.02 \pm 0.02$ ). This implies that when the bacterium swims down the gradient there is no functional relationship between the length of a run and the derivatives of the concentration with respect to space or time. This is true both for serine and aspartate.

There is nothing in our data to suggest that the bacteria are able to steer in the direction of the gradient while running or

that the motion is topotactic<sup>4</sup>. When they twiddle, the change in direction is still biased toward small angles, as in Fig. 3, but the angle chosen does not depend on the direction of the gradient; there is no correlation between the inclination at the end of a run (Table 2, legend) and the change in direction from run to run. Nor is there any correlation between the length of the run and the change in direction.

Table 3 Analysis of Runs which Move the Bacteria Up the Gradient or Down the Gradient

| Attractant                                                                                                   | Serine          | Serine          | Aspartate       | Aspartate       |
|--------------------------------------------------------------------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Net displacement of runs                                                                                     | Up              | Down            | Up              | Down            |
| Mean concentration ( $\mu\text{M}$ )                                                                         | $10.0 \pm 2.8$  | $9.2 \pm 2.6$   | $8.8 \pm 1.9$   | $8.1 \pm 1.9$   |
| Mean run length (s)                                                                                          | $2.19 \pm 3.43$ | $1.40 \pm 1.88$ | $1.07 \pm 1.80$ | $0.80 \pm 1.38$ |
| Mean run length expected from the control run length (Table 2) and the concentration dependence (Fig. 5) (s) | 1.48            | 1.45            | 0.82            | 0.82            |

The runs of the gradient experiments (Table 2) divided into two subsets according to whether the net displacement of a run is toward or away from the mouth of the capillary (up-gradient or down-gradient). The mean speed was only slightly larger for runs up the gradient than for runs down the gradient (2% for serine, 7% for aspartate).

An accurate calculation of the mean rates at which the bacteria drift up the gradients could be made from the information in Tables 2 and 3 if we knew the functional dependence of the mean run lengths (for runs up the gradient) on inclination; the data at hand are inadequate. If we assume that the run-length bias is proportional to  $\cos \theta$  ( $90^\circ < \theta < 180^\circ$ ), the drift rate in serine is about  $2.0 \mu\text{m/s}$ , and the drift rate in aspartate is about  $0.9 \mu\text{m/s}$ . The value for serine is in rough agreement with that obtained by Dahlquist, Lovely and Koshland<sup>15</sup> for suspensions of *Salmonella* in exponential gradients of comparable steepness.

## Mechanisms

When a bacterium runs, its flagellar filaments work together in a bundle of the kind photographed in *E. coli* by Ramsey and Adler (Ramsey, S. W., and Adler, J., private communication), or in *Salmonella* by Mitani and Iino<sup>16</sup>. When it twiddles the bundle probably loosens or comes apart. When the bundle re-forms, the cell goes off in a new direction. The direction chosen depends on the change in orientation of the bundle relative to the body of the cell. Smaller changes in direction require smaller changes in orientation and occur in shorter periods of time (Fig. 3). The stability of the bundle is improved by interaction with chemoreceptors. The association of an attractant with a receptor increases the stability even more. If the attractant is serine, the stability of the bundle is affected by both the average level of association (Fig. 5) and the rate at which it increases (Fig. 6). If it is aspartate, only the rate of increase is important (Fig. 6).

We do not know what the molecular structure of the twiddle generator is or how it is able to perturb the flagellar bundle. We do know it operates on Poisson statistics (Fig. 4) and that its firing rate can be suppressed by chemoreception. It is likely that the generator is built from elements which are missing or defective in generally nonchemotactic mutants. When the generator and the chemoreceptors are uncoupled, the generator runs free, and the mutants are uncoordinated.

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# Random Packing of Equal and Unequal Spheres in Two and Three Dimensions

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**A new computer simulation of random packing of spheres is applied to two- and three-dimensional problems with a uni-directional gravitational force.**

THE ramifications of the sphere packing problem are multifarious. They are found, for example, in metallurgy, ceramics, soil science, biology, physics, chemistry, and many fields of engineering. The problem may be stated as follows: given spheres with radii distributed according to a prescribed probability density, and given that they are packed together randomly by some rule to be specified, what is the nature of the resultant heap? Density, average number of contacts, radial distribution function, and size distribution of interstices are a few of the quantities which have obvious importance in the fields mentioned above and which have been calculated or measured experimentally.

We approach the problem of random packing of spheres by means of a Monte Carlo computer simulation of the physical process of dropping spheres into a bin. Our packing rule differs from previous ones<sup>1,2</sup> in that our spheres position themselves under the influence of a unidirectional (vertical) gravitational force, rather than toward a centre of attraction.

The computer code in three dimensions is set up as follows: In order to avoid difficulties with the sides of an array, periodic horizontal boundaries are assumed, so that a ball at  $(x, y, z)$  reappears at  $(x \pm L_x, y \pm L_y, z)$ . Balls are dropped sequentially from a random point above the  $L_x \times L_y$  bin. When a ball is dropped it hits ball  $m$  or the floor. If it has contacted ball  $m$ , it then rolls down in a vertical plane on  $m$  until it is in contact with  $m$  and  $n$ . Then it rolls downwards in contact with both  $m$  and  $n$  until it makes contact with  $p$ . If the contact with  $m, n$ ,

and  $p$  is stable, it stops. If not, it rolls on the double contact that goes down most steeply, and so on. Any time a ball contacts the floor, it stops.

In two dimensions the code is similar but simpler in that only the discs in the top layer need be checked for hits or contacts.

New features of our calculation include domain structure in 2d random packing, computer simulation of jiggling the container, the effect of machining errors on the buildup of a regular hexagonal close packed (h.c.p.) array of balls, densities of binary random mixtures of spheres in 2d and 3d, and simulation of shape irregularities, interparticle attractions or stickiness.

## Two-dimensional Calculations: Buildup of Heaps of Hoops

The 2d simulation code has been used to build stacks of discs. An example of a binary mixture is shown in Fig. 1. The discs were dropped from random points above the periodic baseline.

Fig. 2 shows an array of discs, monosize except for the bottom layer, which is chosen from a uniform random distribution of radii between  $R=1.0 \pm 0.2$ . This uneven base causes the structure to look quite random for a while, but soon order reappears in the form of domains of nearly square structure, tipped at about  $45^\circ$  from the vertical. These domains are of the order of 8 or 10 discs on a side, and fill most of the stack. The same kind of domain also appears if the array is started with a row of uniform discs with subsequent discs randomly chosen from a uniform distribution with a very small spread like  $R=1 \pm \frac{1}{2} \times 10^{-6}$ ; the smaller the spread the longer the regular hexagonal structure persists. (In the following we call this the fuzzy monosize distribution with spread  $10^{-6}$ .)

These results contrast with those obtained by Kausch *et al.*<sup>3</sup> who found that ordered domains will form spontaneously, but that the ordered domains constituted only a small fraction of

the array, and that the structure within domains was hexagonal. These differences must be ascribed to the fact that they used relatively small arrays, so that the convergence of the central force could not be neglected, while we use a unidirectional gravitational force.

## Density and Simulation of Shaking of the Bin

The density of random stacks of monosize and fuzzy monosize discs is 0.82. The density of the square array which constitutes the domains is  $\pi/4=0.785$ . The density along the domain boundaries, where sometimes neighbouring square arrays mesh with each other, is clearly higher (locally up to  $\pi/\sqrt{12}=0.907$ , the regular close packed density), which must account for the rise in the net density from 0.785 to 0.82.

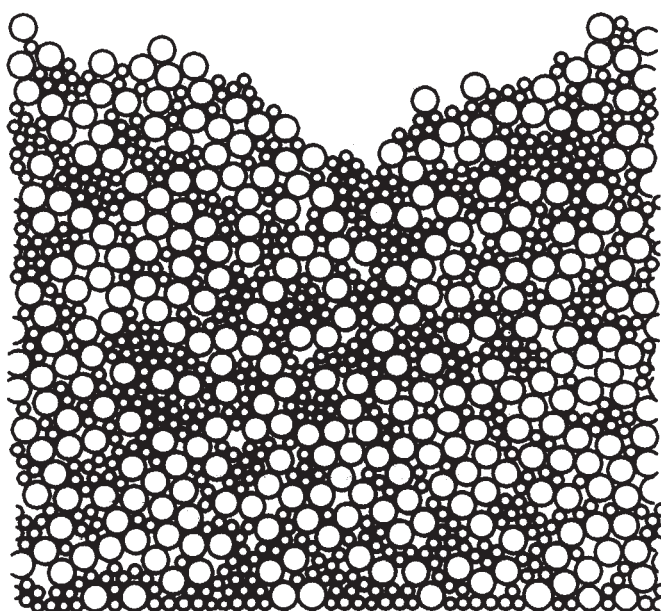


Fig. 1 A pile of discs built up by dropping each from a random position above the baseline. The radius ratio is 2 and 70% of the hoops are small ones.

Shaking the bin can be simulated by changing the rules slightly. Physical shaking in an experiment would be difficult to duplicate with a computer, because many spheres then move simultaneously. Physical shaking increases the density by allowing the structure to seek a configuration of lower potential energy. We form a random structure of lower potential energy in an alternative way as follows. Ordinarily the structure is built up as explained above, by dropping a single disc at a time. If, instead, we drop  $m$  trial discs, where  $1 < m \leq 20$ , but allow only the one whose final position is lowest to remain and become a part of the stack, then clearly the density of the stack will be increased. Because of the ordered nature (tipped square domains) of the random stack, however, in the 2d case the increase is found to be negligible.

## Binary Mixtures

Although our code can use any distribution of disc radii, we have only calculated stacks for monosize, fuzzy monosize, and binary distributions. It is intuitively reasonable that the monosize distribution will yield a minimum density; this is borne out by the numerical results we have obtained for binary mixtures. It is also intuitively clear that the maximum binary

density is obtained in the limit  $r_1/r_2 \rightarrow 0$ , with random arrays of small discs filling the interstices left in a random array of large ones. This yields a density of  $\rho_2 = 1 - (1 - \rho_1)^2$ , where  $\rho_1 = 0.82$ , the monosize random density. (For a ternary distribution, with  $r_1/r_2 \rightarrow 0$ ,  $r_2/r_3 \rightarrow 0$ ,  $\rho_3 = 1 - (1 - \rho_1)^3$ , and so on.) This value of  $\rho_2 = 0.97$  is clearly unattainable in a 2d random array formed by our method, because the stack of large discs is impermeable to the small ones. In 3d the situation is different, because interstitial passages exist between the spheres. In random stacks of binary mixtures the increase in density over a monosize stack is small; the most pronounced feature is a sudden 3 or 4% increase in density when one goes from all small discs ( $f_1 = 1$ ) to a mixture of mostly small discs plus a few large ones. This is not surprising; Kausch *et al.* found a similar effect for the case they computed ( $r_1/r_2 = 5$ ).

## Monosize Spheres

To test the 3d code, we used it to generate a h.c.p. structure. To do this it was necessary to choose the  $y$ -period equal to a rational multiple of  $\sqrt{3}$  times the  $x$ -period, and place the bottom layer carefully in a hexagonal array. The subsequent balls were dropped from positions directly above these base balls, but displaced a certain constant arbitrary amount to fix the direction of the initial roll. This procedure did, in fact, generate a h.c.p. array, provided that the base balls were chosen to have radii very slightly larger than the radii of the subsequent balls, to circumvent roundoff.

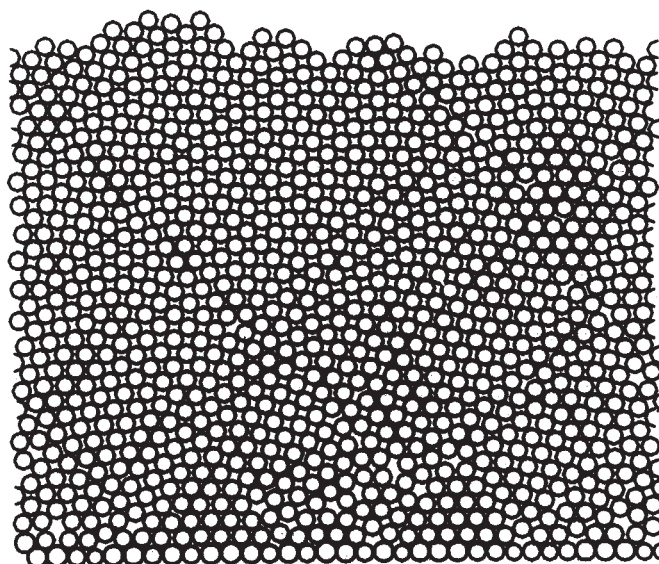


Fig. 2 As Fig. 1 but all discs have the same radius with the exception of the bottom row, which is irregular. Notice that after our initial mixed-up region near the baseline, the structure arranges itself into domains.

Keeping the same base ball field and dropping procedure, we changed the distribution of subsequent balls from monosize to fuzzy monosize with  $10^{-6}$  spread. This had the effect of destroying the regular h.c.p. structure after 7 or 8 layers of balls, with a sudden transition to a random structure with density 0.582. This transition is clearly shown in Fig. 3, which shows density as a function of altitude in the array. Very suddenly after 7 or 8 layers have been built up with the characteristic density ( $\pi/\sqrt{18}=0.740$ ) of the h.c.p. structure, the density drops to 0.582 with relatively small fluctuations. The results are similar for a fuzzy monosize distribution with



$10^{-4}$  spread. Here the larger fluctuations in sphere radii cause the misfitting spheres to build up their displacements from h.c.p. lattice points faster, and only 3 or 4 layers are regular.

The radial distribution function for these arrays of balls has been calculated (Fig. 4). The average number of contacts per ball is computed to be 6.4 (in fair agreement with the 6.7 implied by ref. 2); the discontinuity at  $4R$  is caused by the frequent occurrence of triplets of nearly collinear contacting balls.

## Effect of Changing Sequential Addition Algorithm

As in the 2d case we can stimulate shaking the bin by dropping  $m > 1$  balls at each step, and selecting the one which comes to rest at the lowest stable 3-ball contact. For  $m$  as small as 4 or 5 the density is increased from 0.582 to 0.600; further increase in  $m$  does not result in a larger density. It may be significant that this increase of 0.018 is just  $\frac{1}{3}$  of the increase required to reproduce Scott and Kilgour's density<sup>4</sup> of jigged ball bearings. We are compacting our pile by minimizing gravitational energy, which is manifested by a unidirectional force. Collective rearrangements of slippery ball bearings may effectively be compacting the stack by forces in 3 directions.

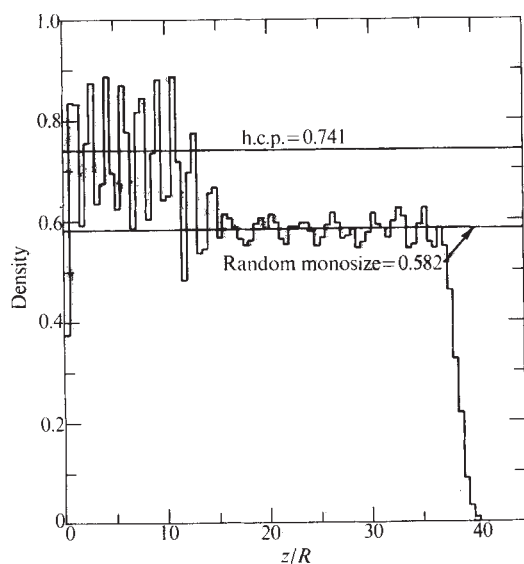


Fig. 3 Densities of stacks of spheres as functions of height above floor of bin. The bottom layer is placed in a regular hexagonal array, and is composed of spheres with radius  $1 \times 10^{-6}$ . Subsequent balls are chosen from a fuzzy monosize distribution of width  $10^{-6}$ .

Effective simulation of stickiness or irregularity of particle shape can be attained by another change of the rules. If we limit the roll of a dropped sphere to its first three-point contact instead of allowing it to roll all the way to a gravitational minimum, then we expect the resultant random structure to be much looser. This in fact is the case; the density drops to 0.38.

## Absence of Domain Structure in 3d

The fact that randomly packed structures in 2d consist mostly of ordered domains suggested the intriguing possibility that a similar feature might exist in the random 3d arrays. Evidence which bears on this question can be gleaned from the data (coordinates of sphere centres in random arrays) in several ways. One might think, in analogy to the fact that domains

have square structure in 2d, that they might have simple cubic structure in 3d. This conjecture is supported by the average number of contacts per ball, 6+, but is vitiated by the absence of a peak at  $2\sqrt{2}R$  in Fig. 4. In fact, the overall smoothness of the radial distribution function is evidence against the existence of domains.

In order to look for domain structure, we program the computer to bisect a random array of balls with a plane of specified altitude, polar angle and azimuth. The plane cuts the spheres; the locus of the intersection of the plane with a sphere will be a circle in the plane, with radius between 0 and  $R$ . For many orientations of the plane, the circles in the section will form an easily recognizable ordered pattern if the spheres form a locally ordered array.

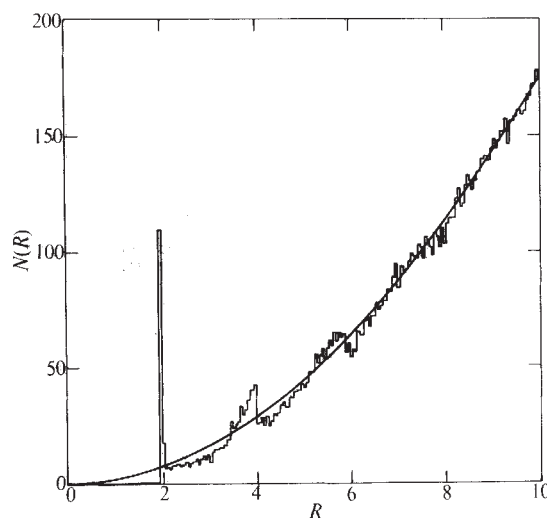


Fig. 4 Radial distribution function for a monosize random array of 2,000 balls, calculated by requiring that one of the balls in a pair be in the middle 20% of the height of the stack, to eliminate boundary effects. The parabola is  $0.5823R^2$ , the radial distribution function for an uncorrelated pile with density 0.582. Note the absence of bumps at  $2\sqrt{2}$ ,  $2\sqrt{3}$ , and  $4\sqrt{2}/3$ , indicating a lack of short-range structure with simple cubic or h.c.p. symmetry. The histogram step here is 0.05.

We have programmed the computer to draw these sections on film for sequences of closely spaced polar and azimuthal angles. These films thus consist of sets of movie frames, showing the sections formed by continuously rotating and tilting the plane. These sets have not shown any locally ordered domains in an overall disordered structure, nor do domains show in "jigged" arrays with  $m=20$ . A sample section from a fuzzy monosize array is shown in Fig. 5. The demarcation between ordered and disordered structure is clearly visible.

## Random Mixtures with Two Different Radii

It is easy to establish a framework to which we can relate densities of binary random mixtures of spheres by expressing the binary density in terms of the monosize density in certain limiting cases. In a filled bin of volume  $V$ , the packing density is

$$\rho = \rho_1 + (1 - \rho_1) \rho_2 \quad (1)$$

where  $\rho_1$  = densities of large balls in  $V$  and  $\rho_2$  = density of small balls within the volume  $(1 - \rho_1)V$  available to them. We define the volume fraction of large balls  $y = \rho_1/\rho$ . Then equation 1 gives  $\rho = \rho_2/(1 - (1 - \rho_2)y)$ . This relation is true for

all binary mixtures and we know  $\rho_2$  in certain limits. First, for  $r_2/r_1 = r \rightarrow 0$  clearly  $\rho_2 = 0.582$  in the unshaken case, and  $\rho = 0.582/(1 - 0.418 y)$  if there are enough small balls to fill up all the interstices in a random array of large balls. For  $y > 0.705$  there is a deficiency of small balls and one finds  $\rho = 0.582/y$  if one assumes that the small balls distribute themselves uniformly throughout the volume available to them. Fig. 6 shows these limits plotted together with an even more trivial one for  $r = 1$ , for which the density is independent of  $y$ . Also shown are some results of our computer simulation of binary densities. These points, for radius ratios  $r$  of 4 and smaller, are consistent with the above limits and with the reasonable conjecture that the density increases monotonically as the two radii become more disparate. To achieve densities near the upper limit of 0.825, it is clearly required to go to very small  $r$ , for which the mixture will contain hundreds or even thousands of small balls for each large one. Our computer cannot reasonably handle such a situation. Even for the modest  $r = 0.25$  point at  $y = 0.76$  there were 20 small balls in the array for each large one.

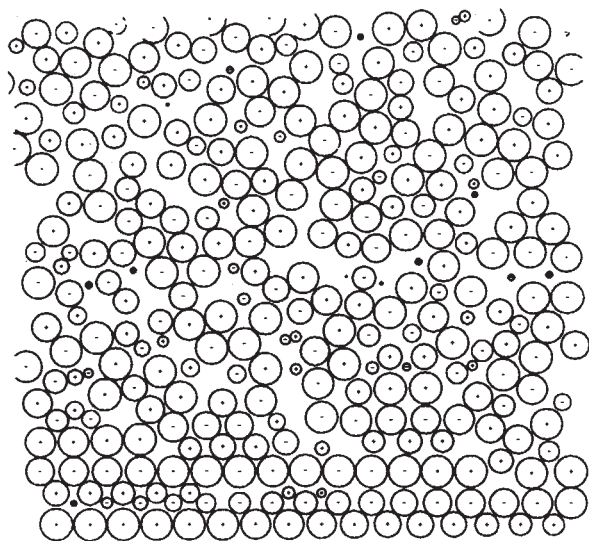


Fig. 5 Section cut from a fuzzy monosize array with width  $10^{-3}$ . A plus at the centre of a circle means the centre of the corresponding sphere lies above the page, a minus means below.

## Other Radius Distributions

Our calculations, insofar as they have been at all systematic, have been limited to monosize and binary distributions. Our computer code will take any distribution whatever, but the calculation, for reasons of memory and time, is limited to total numbers of balls less than about 10,000. Although this number seems large, it is not large enough to generate an array with good statistics even for  $r = 0.1$  in the binary case, let alone simulate an array which is close to one of practical interest to ceramicists or metallurgists, that is, one with a continuous radius distribution with radius disparities of 100 or 1,000. These latter must be approximated, in our opinion, by analytic means; some attempts in this direction have been made<sup>1,5,6</sup>.

## Other Systems

The best studied sphere packing problem experimentally, theoretically, and numerically, is the compacted stack of equal slippery spheres. Part of the reason it has attracted such a lot of attention is that it is easiest; but also it may be the array

which a hard sphere liquid seeks in the limit of zero temperature and/or infinite pressure. This has been pointed out by LeFevre<sup>7</sup> who has plotted the results of several numerical statistical mechanical calculations of the fluid branch of the equation of state of a gas of hard spheres and shown that they point toward a zero temperature intercept very close to the Scott-Kilgour density.

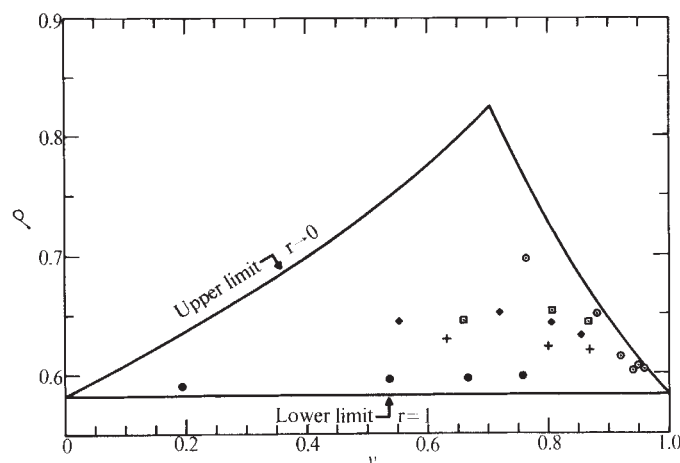


Fig. 6 Densities of binary mixtures of spheres as a function of volume fraction of large balls for various radius ratios  $r = r_2/r_1$ . Only the regions where  $y$  and/or  $r$  are fairly large are at present accessible to Monte Carlo calculations, because of time and memory limitations.  $\circ$ ,  $r = 0.25$ ;  $\square$ ,  $r = 0.30$ ;  $\diamond$ ,  $r = 0.35$ ;  $+$ ,  $r = 0.40$ ;  $\bullet$ ,  $r = 0.60$ .

If this is more than an accidental coincidence it serves to emphasize the desirability of finding a computer algorithm to generate the Scott-Kilgour stack to enable one to analyse it for short-range order and domain structure. As far as we know such an algorithm is as yet undiscovered; that of Norman<sup>1</sup> and Adams and Matheson<sup>2</sup> in fact seems to generate a pile with the same densities as our "shaken stack" further than about 4 diameters from the centre of attraction. That is, the packing density as a function of distance from the centre  $R$  (see Fig. 4, curve f of ref. 2) can be closely fitted with a model which has a uniform density of 0.60 for  $R > 4D$  and an average density of 0.64 for  $R < 4D$ . So, as one would expect, for large enough  $R$  their rule yields the same result as ours, and neither gives the Scott-Kilgour density. The radial distribution function of Adams and Matheson shows, unlike ours (Fig. 4) but in agreement with that of Scott<sup>8</sup>, a bump at or near  $\sqrt{3}D$ . This we interpret to mean that perhaps Adams and Matheson's structure<sup>2</sup> is close to ball bearings near the centre of their spherical array.

A more detailed version of this work is available<sup>9</sup>. One of us (W. M. V.) thanks H. D. Lewis and A. Goldman, especially the latter, for encouragement.

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<sup>5</sup> Blum, E. H., thesis, Princeton Univ. (1965).

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<sup>7</sup> LeFevre, E. J., *Nature*, **235**, 20 (1972).

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<sup>9</sup> *Los Alamos Report*, LA-DC-72-745.



# LETTERS TO NATURE

## PHYSICAL SCIENCES

### Bode's Law and the Missing Planet

EXACTLY two hundred years ago, Titius<sup>1</sup> published a mnemonic for the mean distances of the planets from the Sun. His rule was

$$a_i \propto 2^{(i-2)} \cdot 3 + 2^2$$

where  $a_i$  is the major semi-axis of the orbit of the  $i$ th planet from the Sun. Titius's law represents the distances of the then known planets with an accuracy of a few per cent, provided that (i) for Mercury, we take  $i = -\infty$  instead of  $i = 1$  and (ii) the orbital  $i = 5$  is left vacant. The law made three valid predictions. Uranus (discovered by William Herschel in 1781) fits the orbital  $i = 8$ . After the discovery of Uranus, Bode publicized the law, which became known as Bode's Law. The search for a planet for  $i = 5$  culminated in the discovery of the first asteroid, Ceres, in 1801. Recognition of a similar law for the satellite system of Saturn led to the discovery of Hyperion in 1848.

While no doubt Bode's law as it stands is not of precise physical significance, it has long been asked whether the distribution of planetary (and satellite) orbits is due to chance, or to some physical principle that would operate in any planetary system. Possibilities for such a physical process are (i) properties of the mechanism of formation of the solar system, (ii) mutual planetary perturbations subsequent to the formation of the solar system, such perturbations being either the simple point-mass gravitational perturbations, or else perturbations involving dissipative forces (such as tidal action).

I have shown that the point-mass perturbations (without dissipative forces) are a sufficient explanation of the existing distribution of satellite and planetary orbits, affording a reason for the present planetary distances to an accuracy  $\sim 1\%$ . The following is a brief summary of my arguments. They will be published in detail elsewhere (refs. 2, 3 and Feagin, Graf and M. W. O., unpublished results).

The intuitively obvious conclusion that a set of planets will rapidly change their configuration when their mutual interactions are strong—and will change only slowly when their interactions are weak—is confirmed by numerical integrations of simulated satellite systems<sup>2-4</sup>. I generalize this result into the "Principle of Least Interaction Action".

A satellite (or planetary) system of  $N$  point masses moving under their mutual gravitational attractions will spend most of its time close to a configuration for which the time-mean of the action associated with the mutual interactions of the satellites is an overall minimum.

The condition for minimum interaction action is simply the condition that the time-mean of the disturbing function

$$R = \sum_i \sum_j \frac{m_i m_j}{\rho_{ij}} \quad (j > i)$$

is a minimum, where  $m_i$  is the mass of the  $i$ th satellite (relative to the primary) and  $\rho_{ij}$  is the instantaneous separation of the  $i$ th and  $j$ th satellites. The theorem of Poincaré that a resonant gravitational system has the property that the disturbing function, averaged with respect to the critical parameter, has a local minimum, leads immediately (from the Principle of Least Interaction Action) to the two-body resonant structures<sup>5,6</sup> shown to be preferred in the solar system, and explained in a

manner wholly consistent with the present arguments. I ask whether the local minima are also overall minima.

To test the Principle of Least Interaction Action, it is necessary to know the configuration of minimum interaction action available to any system of satellites. Because we cannot, as yet, integrate the equations of motion numerically for times of the order of  $10^9$  orbital periods, I have made a number of approximations in order to deduce an approximate sequence of configurations for a system. First, I assume (for calculating the evolutionary sequence) that the masses of the satellites are negligible compared with the mass of the primary, although I still seek a minimum of  $\bar{R}$ . Second, I assume that evolution proceeds through a sequence of quasi-circular orbits (which assumption must be true to some degree of accuracy for some length of time before the present epoch, since most of the orbits in the solar system have small eccentricities). Third, I estimate the energy available, at any time, to drive a secular change through the computed evolutionary sequence from the fact that, in the equation of motion for a perturbed planet, expanded in series, secular terms first appear in terms  $\sim em^3$ , where here  $m$  is the mass of the perturbing planet relative to the primary, and  $e$  the orbital eccentricity. In this way I can both locate the configurations of minimum interaction action to the accuracy of my assumption of circular orbits and also estimate the evolutionary time-scales of the systems considered.

The following results have been obtained, which confirm the essential validity of the techniques used. (i) The present distribution of the five satellites of Uranus is within 5% of the calculated minimum interaction action distribution, with the exception of the innermost body, UV, whose mass is given to only one significant figure. The discrepancy can be reduced by increasing the mass of UV. The time-scale is  $\sim 2 \times 10^9$  yr. (ii) The five inner satellites of Jupiter form a quasi-isolated system. Their present distribution is also within 5% of the distribution of minimum interaction action, except again for the innermost satellite, JV. In this case no mass at all is tabulated for JV, and the assumed mass of  $10^{-6} \times$  the mass of Jupiter may well be wrong. The evolutionary time-scale is  $\sim 6 \times 10^7$  yr. (iii) The triplets UV, UI, UII and JI, JII, JIII show the Laplace relationship between the mean motions

$$\begin{aligned} \text{Jupiter } n_1 - 3n_2 + 2n_3 &= n_1(2 \times 10^{-7}) \simeq 0 \\ \text{Uranus } n_5 - 3n_1 + 2n_2 &= n_5(3 \times 10^{-4}) \simeq 0 \end{aligned}$$

The slower evolution of the Uranian system is the reason for the larger proportional discrepancy from zero of the resonant relationship. The time required to approach within the observed precision of resonance is  $2 \times 10^9$  yr for the Jovian system and  $6 \times 10^9$  yr for the Uranian system. It is remarkable that two systems, for which the relative discrepancies differ by four orders of magnitude, should not merely give the same evolution time to better than an order of magnitude but that this time should be the age of the solar system, determined from meteorites to be  $4.5 \times 10^9$  yr.

I now apply the technique to the planetary system, taking first the system of Jupiter, Saturn, Uranus and Neptune (JSUN)\*. Line 4 of Table 1 shows the minimum interaction

\* Pluto is not considered here. It is not massive enough appreciably to affect the critical distribution of JSUN (Nacozy and Diehl, private communication) and if the orbits of Jupiter, Saturn, Uranus and Neptune are included in the Pluto system with fixed orbits, Pluto is in a configuration corresponding to minimum interaction action.

action configuration for this system. Line 3 shows the present major semi-axes. The discrepancies are large, despite the fact that the time-scale for evolution of this system to minimum  $\sim 5 \times 10^8$  yr.

The discrepancies may be precisely removed if it is assumed that material (referred to as A) of mass  $90 \pm 5 M_{\oplus}$  (where  $M_{\oplus}$  is the mass of the Earth) had been in the asteroid belt from the beginning of the solar system until  $1.6 \pm 0.4 \times 10^7$  yr ago, and that it then dissipated. Since then, the JSUN system has been evolving towards its new minimum interaction action distribution. Line 1 of Table 1 shows the minimum interaction action distribution of the system AJSUN, which it would have reached on a time-scale  $\sim 5 \times 10^8$  yr. Line 2 of Table 1 shows the configuration that JSUN had  $1.6 \times 10^7$  yr ago, as found from my evolutionary sequence.

**Table 1** Major Semi-Axes (A.U.) of Outer Planets

| A     | J     | S     | U     | N     |                                             |
|-------|-------|-------|-------|-------|---------------------------------------------|
| 2.794 | 5.211 | 9.509 | 19.46 | 29.71 | Minimum config.                             |
| —     | 5.206 | 9.506 | 19.40 | 29.74 | $-1.6 \times 10^7$ yr                       |
| —     | 5.203 | 9.539 | 19.18 | 30.06 | Present $t=0$                               |
| —     | 5.15  | 10.5  | 12.7  | 37.3  | Minimum config.<br>( $+2.8 \times 10^8$ yr) |

$M_A = 90 M_{\oplus}$ .

The time-scale for the evolution of the residue of A (taken to be  $0.1 M_{\oplus}$ ) presently in the asteroid belt is such that changes of only 0.05 A.U. are to be expected in  $10^7$  yr. The fact that the minimum configuration has A close to the present mean major semi-axes of the asteroid belt is an additional piece of evidence of the internal consistency of the interpretation. It should be noted that the sequence of minimum configurations as a function of  $M_A$  and the evolutionary sequence of JSUN are both one-parameter systems, so a match of five separate quantities to a precision  $\sim 0.3\%$  is very unlikely by chance. Not only that, but the time found for the dissipation of A is a physically-meaningful time, being in reasonable agreement with the longest cosmic-ray shielding times for chondrite and achondrite meteorites. The picture is therefore consistent with the break-up of a single body into pieces at this epoch, some already below the critical cosmic-ray shielding size, others breaking to that size by subsequent collisions.

The confirmatory evidence of the former presence of A comes from considering now the system of the terrestrial planets Mercury, Venus, Earth and Mars from Jupiter. If the hypothesis is correct, the present positions of MeVEMsJ should correspond to the evolution, through  $1.6 \times 10^7$  yr, of this system from the minimum interaction configuration of MeVEMsAJ since the evolution time of MeVEMsAJ  $\sim 10^9$  yr. In fact, the characteristic time of evolution of the MeVEMsJ system  $\sim 8 \times 10^{11}$  yr, so that effectively the present positions of MeVEMsJ should correspond to the minimum interaction action distribution of MeVEMsAJ. The agreement shown in Table 2 is good. The major discrepancies are for the planets Mercury and Mars, which have high eccentricities (0.205 and 0.093 respectively). The errors are thus no larger than would be expected from the approximations made in calculating R for an elliptical orbit as though it were a circular orbit.

**Table 2** Axes of Inner Planets

|                 | Me    | V     | E    | Ms   | A    | J    |
|-----------------|-------|-------|------|------|------|------|
| Minimum config. | 0.394 | 0.719 | 1.00 | 1.49 | 2.79 | 5.20 |
| Present config. | 0.387 | 0.723 | 1.00 | 1.52 | —    | 5.20 |
| Minimum config. | 0.461 | 0.696 | 1.02 | 1.24 | —    | 5.21 |

$M_A = 90 M_{\oplus}$ .

I claim that the approximate treatment described above is adequate to deal with the problem, and that the agreement of observation with prediction and the complete internal

consistency of the evolutionary picture give compelling reasons for accepting the essential correctness of the hypotheses put forward.

Two major problems remain. First, what physical process caused the sudden dissipation of A? From the point of view of the dynamical arguments presented here, it is probably true that A was always in the form of a ring. But while it may be difficult to "explode" a planet, it would seem even more difficult to dissipate a ring suddenly after it has been quiescent for  $4.5 \times 10^9$  yr. Second, only  $\sim 0.1 M_{\oplus}$  seems now to reside in the asteroid belt. What has happened to the other  $89.9 M_{\oplus}$ ?

I thank the University of British Columbia for the granting of a year's study leave, and the Institute of Theoretical Astronomy, Cambridge, the Department of Aerospace Engineering and Engineering Mechanics of the University of Texas at Austin, and El Instituto de Astronomia de la Universidad Nacional Autónoma de México for their hospitality. The work was supported in part by a grant from the National Research Council of Canada.

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## Daytime Laser Radar Measurements of the Atmospheric Sodium Layer

It is generally accepted that the column density (abundance) of atomic sodium in the layer at about 90 km altitude is enhanced during the day by a factor of 2 to 6 relative to that at twilight. The evidence for the enhancement is chiefly provided by ground-based observations of the sodium D line dayglow<sup>1-5</sup>, but there has been some discussion<sup>4-6</sup> of the possibility that the ring effect (the partial filling of the Fraunhofer lines in scattered sunlight) may have affected the measurements. Observations of the dayglow during 6 rocket flights<sup>7-9</sup> and spectrometer observations of the absorption of direct sunlight by sodium<sup>10-12</sup> have produced various conflicting results concerning the daytime enhancement. Recently, however, Burnett *et al.*<sup>13</sup> have published the results of absorption measurements at latitude  $26^\circ$  N giving daytime abundances which, when compared with twilight measurements made at a similar latitude some years previously, are clearly much lower than would be expected if the enhancement were present. In an independent effort to resolve the question of the daytime enhancement we have developed further the technique of resonance scattering from a laser pulse tuned to the D line<sup>14</sup> to measure the sodium concentration during the day. Our previous measurements<sup>15,16</sup> have shown that the night-time abundance is within 20% of that at twilight and we now find no evidence of a daytime enhancement.

The night-time laser radar system (Table 1) is unsuitable for measurements during the day because the daytime sky would produce a photon count rate of up to 2 GHz. That is far in excess of the maximum synchronous count rate of the photo-multiplier system, 30 MHz, and the ratio of the signal counts to the background counts would be very small ( $\sim 10^{-4}$ ). For the daytime system, therefore, we used a different receiver



**Table 1** Optical Radar System

|                                                                              |                    |                    |
|------------------------------------------------------------------------------|--------------------|--------------------|
| <b>Transmitter</b>                                                           |                    |                    |
| Wavelength                                                                   | 589.0 nm           |                    |
| Linewidth                                                                    | 3 pm               |                    |
| No. of photons transmitted (for one 200 mJ laser pulse)                      | $2 \times 10^{17}$ |                    |
| Beam width (containing all the transmitted photons)                          | 0.3 mrad           |                    |
| Pulse duration                                                               | 1 $\mu$ s          |                    |
| Pulse repetition rate                                                        | 2 s <sup>-1</sup>  |                    |
| <b>Receivers</b>                                                             |                    |                    |
|                                                                              | Night-time         | Daytime            |
| Mirror area                                                                  | 0.6 m <sup>2</sup> | 0.3 m <sup>2</sup> |
| Beam width                                                                   | 0.7 mrad           | 0.3 mrad           |
| Separation from transmitter                                                  | 2 m                | 3 m                |
| Bandwidth                                                                    | 1 nm               | 50 pm              |
| Overall efficiency                                                           | 1%                 | 1%                 |
| <b>Data recording</b>                                                        |                    |                    |
| 30 range-gated counters normally read-out after accumulating 200 laser shots |                    |                    |

channels were calibrated to better than 0.1% accuracy by observing the counts with the laser not firing. To prove that the daytime receiver was operating correctly it was used at night to measure the sodium density with a source of background light of variable brightness alternately switched on and off for runs of 200 laser shots. The sodium density measured by the receiver was found to be independent of the background light level over the whole range of brightness between night and noon in summer.

As in the previous night-time measurements, sodium densities were calculated from the ratio of the signal from the sodium layer to that from Rayleigh scattering in the 25–35 km region of the atmosphere. Because of the small beam divergence and appreciable separation between the transmitter and the daytime receiver, a slight misalignment between the transmitter and receiver beams would affect the ratio of signals from different heights and thereby alter the calibration by a constant factor.

**Table 2** Day/Night Abundance Ratio

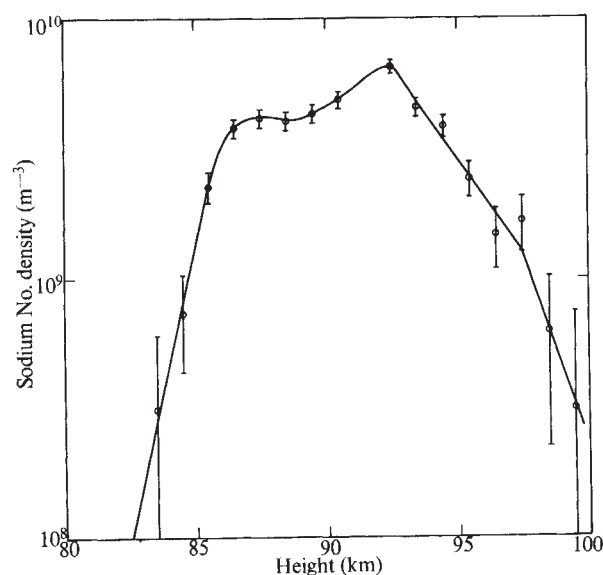
| Date                | Time of observations (UT) |           | Mean abundance during day   | Mean abundance within 3 h of noon |
|---------------------|---------------------------|-----------|-----------------------------|-----------------------------------|
|                     | Day                       | Night     | Mean abundance during night | Mean abundance during night       |
| October 6, 1971     | 1300–1800                 | 1800–2200 | 1.06                        | 0.82                              |
| October 7, 1971     | 1300–1800                 | 1800–2200 | 1.10                        | 1.04                              |
| November 2, 1971    | 1100–1700                 | 1700–2200 | 0.97                        | 0.95                              |
| November 14, 1971   | 1200–1300                 | 1900–2100 | 1.42                        | 1.42                              |
| July 12, 1972       | 1800–2100                 | 2100–2400 | 0.95                        | —                                 |
| July 13, 1972       | 0500–0900                 | 0000–0300 | 0.92                        | —                                 |
| July 15, 1972       | 1100–2100                 | 2100–2300 | 1.04                        | 0.82                              |
| Mean over all dates |                           |           | $1.07 \pm 0.06$             | $1.01 \pm 0.11$                   |

(Table 1), which reduced the background by a factor of 200 and improved the signal-to-background ratio by a factor of 100, with the same dye laser<sup>17</sup> in the transmitter. The signal due to scattering of the laser beam at any height was deduced by subtracting the average background given by the counts observed in the channel corresponding to 160–240 km height where no laser signal was expected. To avoid large errors when the signal-to-background ratio was small, the widths of the

This factor was determined by making simultaneous measurements with both receivers at night.

During the observing periods the laser was normally operated for 4 runs of 200 shots each hour. Fig. 1 shows an example of an autumn height distribution averaged over about 1 h. No systematic difference has been found between daytime and night-time distributions but it is possible that a small difference could be masked by short-term fluctuations. For the least accurate height distribution, obtained at noon in summer, the error bars were 2.5 times larger than those shown in Fig. 1. Even so, the largest fractional error in the small abundances that were observed in summer was only 20% for a one-hour average at noon and the error was considerably less at other times of day. Thus it was sufficiently small to enable accurate day/night ratios of abundance to be determined. For each date when observations were made both by day (solar zenith angle < 96°) and by night (> 96°), the day/night ratios, derived using the observations throughout the day or only observations within 3 h of midday, are given in Table 2. These results show clearly that there is no regular daytime enhancement at our site (51° N). It has been noted above that the results of Burnett *et al.*<sup>13</sup> at latitude 26° N are inconsistent with an enhancement of the magnitude indicated by the dayglow measurements. It therefore seems that the daytime enhancement is confined to the range of latitudes (30° N–45° N) where the dayglow photometer measurements have been made. Alternatively, there may be no daytime enhancement if, for some reason, the dayglow measurements have not yielded true abundances, for example if they have been affected by the ring effect.

The absence of the daytime enhancement would remove one of the difficulties in the development of an overall theory of the sodium layer, because no satisfactory explanation of the enhancement has been proposed. Further observations should be made to look for minor diurnal changes in the height distribution which might be expected to occur. To measure the extremities of the layer more accurately, it would be desirable to improve the signal-to-background ratio in our present



**Fig. 1** Height distribution for 1,000 laser shots between 1205 and 1257 UT, October 27, 1971. The bars are the standard errors due to the limited photon count. The absolute density scale is uncertain to  $\pm 30\%$  because a night-time calibration was not possible on this date.

equipment by about a factor of 50. Because of the effect of the Fraunhofer line this could be achieved by reducing the receiver bandwidth by a factor of 5, to 10 pm, which is technically feasible.

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## Possible Use of Stonehenge

HAWKINS<sup>1,2</sup> has suggested that the Aubrey holes at Stonehenge were used to count the years of a 56-year cycle, important in the motion of the Moon and the occurrence of eclipses. Hoyle<sup>3</sup> and Colton and Martin<sup>4</sup> have suggested other ways that Stonehenge could have been used to predict eclipses. Here we suggest a use of Stonehenge that has nothing to do with eclipses. We suggest also that the Aubrey holes, if they were used as counters at all, were used to count intervals of 56 months rather than 56 years.

In accounting for the Aubrey holes, we have considered only the original structure at Stonehenge, suggested by Atkinson<sup>5</sup> to have been (a) the bank and ditch, which formed a circle broken by the entryway, (b) the 56 Aubrey holes named for their modern discoverer, which form a circle just inside the ditch, and (c) the Heel Stone, which marks approximately the point of Sunrise at the summer solstice as seen from the centre of the circle. There may also have been simple structures to give formal definition to the entrance and to the centre. Stonehenge probably retained this simple structure for about 150 yr, until it was altered by a newly-arrived people.

Thus, to explain the Aubrey holes, we should use only the Heel Stone. An explanation based on eclipse cycles seems unlikely because of visibility problems. Roughly speaking, one lunar eclipse out of two is visible at a particular spot and one solar eclipse out of six or seven. Eclipses unobserved through bad weather are a great hazard in trying to find eclipse cycles; Babylonian and Greek astronomers probably did not discover even the simplest cycle<sup>6</sup>, much less the 56-year one, until more than a millenium after the Aubrey holes were dug.

About a third of the world's population uses a lunar calendar today, and the fraction was probably greater in ancient times. Thus it is plausible that the "Stonehengers" used a lunar calendar if they used one at all. Ancient peoples who used a

lunar calendar often determined the beginning of the month by direct observation of the Moon. It was common to begin a lunar month with the first Sunset at which the new Moon was visible in the west, and it was common to have an official charged with making this observation.

The new Moon sets soon after the Sun; the observer could easily note the relation of the points of Moonset and Sunset. An observer charged with watching the new Moon each month should soon notice that it sometimes sets northward from the Sun and sometimes southward. Since the observer at Stonehenge was presumably also concerned with the summer solstice, he might easily become concerned with the setting of the new Moon that came next after the summer solstice. We will call this the summer new Moon, and consider the time at which the summer new Moon crosses the summer Sun with regard to their setting points.

When this occurs, one of the two nodes of the lunar orbit must be near the celestial position of the summer solstice. After 223 lunar months (= 18 yr and 11 day), the position of the mean Moon is 10.8° east of where it started and the position of the node is 11.3° east of where it started. Thus the Moon is at almost exactly the same position with respect to the node and the node is still close to the solstice. If the summer new Moon crosses the Sun from, say, south to north at the beginning of a 223-month period, it will do so again at the period. However, at either 111 or 112 months, depending on the exact phases of the motion, it will cross in the opposite direction.

The numbers in the preceding paragraph are the ones that occur most often. Because none of the fundamental periods involved is commensurate, all simple cycles fail within a few repetitions. In order to test this cycle, we calculated 12 successive intervals between crossings of the summer new Moon, and found intervals of 111 and 112 months four times each, and the interval was lengthened by 12 months in the other four times.

We can easily imagine that the Stonehengers paid attention to the setting of the summer new Moon (or to some other phase; all such phases are governed by the same basic periods). For instance, they might have said that the Moon is dominant when the summer new Moon sets north of the Sun, and they might have said that the Sun is dominant when the summer Sun is to the north of the summer new Moon. We can also easily imagine that such periods had great importance in astrological or other matters and that the Stonehengers might have been greatly interested in predicting when such periods would end.

They could easily have made these predictions with the aid of a counting circle of 111 holes. If they preferred not to build a circle with this many holes, they could have used a circle of 56 holes just as easily. The doctrine would be: when the counter reaches the 55th hole on the second time around, note first whether this is the summer new Moon; if it is not, wait for the next new Moon. At the summer new Moon, whichever one it is, there are two possibilities: either the period is complete or it will be complete at the next summer new Moon. Whether the period is complete now or next summer could have been attributed to superhuman powers. This use of the Aubrey holes involves observations that were probably made each month, and it does not require any azimuth marker except the Heel Stone. It does not involve a complex scheme of counting.

It does not follow that the Aubrey holes had astronomical significance even if we assume that the Heel Stone did. We note the following points. (1) The Aubrey holes have dimensions of several feet, and they vary in both depth and diameter by factors of two or more. This suggests to us that they were not designed as members of a uniform series, as we should expect if they were intended as counters. They also vary in their use as receptacles for cremations. (2) The area around Stonehenge has many circles of about the same age as Stonehenge. Many of them have no apparent astronomical significance. For example, Avebury has a large ditch with four



entrances, and with a circle of markers just inside the ditch. However, the azimuths of its entrances are approximately 70°, 165°, 250°, and 330°. (3) The number of positions in the circles diagrammed by Atkinson varies from 8 to about 100. We noticed no instance of 56 positions except Stonehenge, nor did we notice any number that seemed to be favoured. This suggests that the number was purely a matter of convenience to the builders. (4) The holes in the entryway to Stonehenge had unique positions and may have been looked upon as distinct from the others. That is, the number to be explained, if any, may be 54 or 55 rather than 56. (5) The significance, if any, may be social rather than astronomical. For example, perhaps there were 56 families, clans, or other social units who built Stonehenge and who were entitled to dig one of the Aubrey holes and to inter cremated remains therein if they wished. This could account for the variability in the size and use of the holes. (6) Finally,  $56 = 7 \times 8$ . Both factors occur frequently in numerology and need no explanation specific to Stonehenge.

There are many non-astronomical but plausible explanations for the number of Aubrey holes. If the holes were used to count an astronomical interval, that interval may have been in months rather than years. The use in counting months could arise simply, with no astronomical apparatus beyond a lunar calendar and a marker for the summer solstice. No observing or recording of eclipses would have been required.

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## The Suess Calibration Curve and Archaeological Dating

Suess<sup>1</sup> was the first to publish an extensive calibration curve, based on dendrochronology, relating radiocarbon dates to absolute time lapse, a procedure made necessary by fluctuations in the concentration of <sup>14</sup>C in the Earth's atmosphere over the last few millennia. Other calibrations followed<sup>2-5</sup>, but Suess's revised curve<sup>6</sup> is still the most widely used by archaeologists (*cf.*<sup>7</sup>). It contains a number of re-entrant kinks or "wiggles", which give from two to seven possibilities when it is used to derive an absolute date from a "conventional" <sup>14</sup>C date, in some regions of the curve. Other curves<sup>2,5</sup> also contain one major and several minor wiggles.

Dendrochronological calibrations have been used to reassess particular samples<sup>8</sup> or time relationships between cultures<sup>9,10</sup>, but archaeologists have avoided routine application of the Suess curve because of the difficulty of quoting multiple possibilities in terms of a single probable date together with a standard deviation. A recently proposed method<sup>11</sup> of treating groups of <sup>14</sup>C dates in terms of the inter-quartile range overcomes this difficulty. As Suess strongly supports the absolute validity of the wiggles<sup>5</sup> multiple intersections of the curve may be treated as if they were discrete

fractional probabilities for a single date, and can easily be used in determining the group quartiles (for details see ref. 11).

Using this technique we studied the effects of correcting two series of dates belonging to the European Neolithic for Central Europe<sup>12</sup> and Eastern Europe<sup>13</sup>. Cultures flourishing earlier than the present limit of the bristlecone-pine calibration (*ca.* 6300 "Libby" yr) had to be omitted. A selection of the cultures is shown in Fig. 1.

The most noticeable change produced by the correction is that the dates of several cultures that were previously symmetrically distributed, for example Corded Ware, Vlaardingen and Ezero, now have a markedly skew distribution, and several other already skew distributions have become even more asymmetrical, for example, Pit Comb Ware, Cortaillod, Michelsberg, Schussenried, Late Linear Pottery, Vinča-Pločnik and Boian. Only in three instances is the distribution more symmetrical after correction, namely, Bell Beaker, Funnel Beaker and Butmir. As there is no obvious reason why archaeological cultures should be symmetrically distributed in time, the connotation of mean and standard deviation is hardly applicable<sup>14</sup>. The term "culture" applied to the data of Fig. 1 refers to a set of dates from a single horizon at a single site, to which the extended Central Limit Theorem may perhaps be applied, and to data from several culturally closely related sites. In the latter a slow growth in intensity could be followed by a limited *floruit* and a slow decline, but this subjective assessment is not a suitable basis for judging the significance of the asymmetrical distributions (Fig. 1).

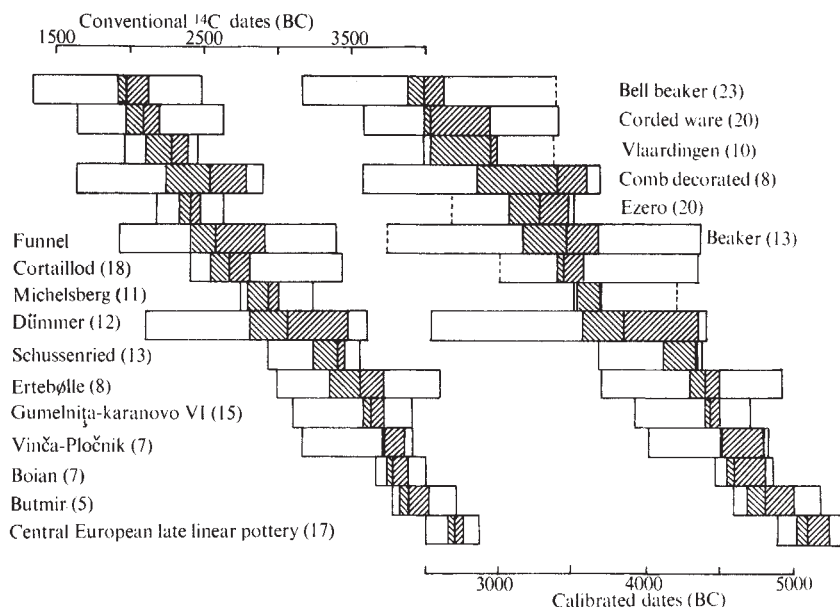
The asymmetries, however, are all associated with a sharp change in slope of the Suess curve (Fig. 2a); if a smoothed curve had been used they would not have appeared. For example, the moderately symmetrical Corded Ware and Vlaardingen distributions whose median dates (uncorrected) are less than 200 yr apart, are changed into two highly asymmetrical distributions which are skew in opposing senses, with median (corrected) dates 400 yr apart, because of a flattening of the Suess curve around 2000 BC and 2300 BC (conventional <sup>14</sup>C dates) which pushes the corrected dates together, and an almost vertical gradient between 2100 and 2200 BC which spreads them apart. This "concertina effect" runs through the series of dates in Fig. 1; the inter-quartile range of several cultures (for example, Michelsberg Ertebølle) has been compressed, while that of others has been extended, sometimes by 2-300 yr, that is, 8-9 generations.

Fig. 3 shows detailed changes in an extreme case. The conventional <sup>14</sup>C dates are symmetrically, if not normally, distributed around a median date of 2250 BC. In the lower part of Fig. 3, considered as a sketch of a probability density function, the distribution has become almost bimodal, with very high probabilities of finding a date at either 2950 BC or 3360-3390 BC, and a low probability in between. These samples were all taken from a single stratum, only 1.75 m thick, of a reliably excavated Bulgarian tell<sup>15</sup>, which was divided into 10 house levels, some of them only 15 cm thick. The corrected dates for each of the five levels from which the samples were taken have a range of possibilities of at least 400 yr—two of them over 800 yr. In our opinion this spread, caused by an almost vertical rise of the Suess curve at 2400 BC (conventional <sup>14</sup>C dates), is unacceptable. The bimodal split of the Corded Ware culture is almost as great. It should be stressed that these effects are not due to the re-entrant wiggles, but to the "staircase" profile of the Suess curve (Fig. 2b).

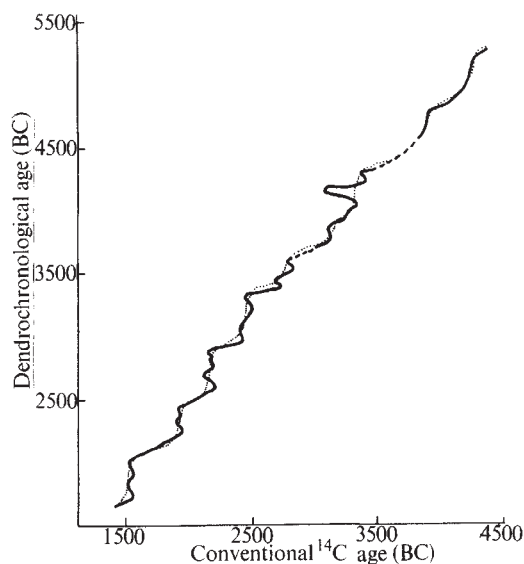
This evidence does not prove conclusively that the Suess curve is inapplicable to archaeological data, and that a smooth curve<sup>4,16</sup> is to be preferred. The floating tree ring sequence from Auvernier<sup>17</sup> provides evidence for wiggles (see also ref. 19). Reservations have been expressed about the existence of the wiggles<sup>16</sup> and about their number<sup>19</sup>, but many workers in this field<sup>2,20</sup> believe that there have been short-term fluctuations in the specific activity of atmospheric <sup>14</sup>C.

Archaeological dating should be directly related to the dendrochronological calibration curve, rather than to a

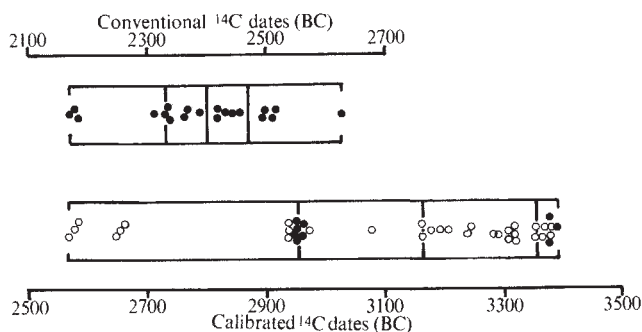
**Fig. 1** Dispersion diagram of the  $^{14}\text{C}$  dates for several Neolithic cultures, from Steuer, Steuer and Tempel<sup>12</sup> and Tringham<sup>13</sup>. Left-hand bars, conventional  $^{14}\text{C}$  dates (upper scale); right-hand bars, dates corrected from Suess curve<sup>6,7</sup> (lower scale). The median and quartile dates are shown, and the inter-quartile range is shaded. A dotted vertical line means that the extreme date is a fractional probability arising from an ambiguity in the calibration curve. The number of dates for each culture is shown in parentheses.



secondary curve smoothed on the grounds that samples from mature wood give an average over several decades. In the Neolithic, for example, many of the samples relate to a single



**Fig. 2** —, Bristlecone-pine calibration curve published by Suess<sup>6</sup>. . . ., Smoothed curve drawn by hand through Suess's curve, which retains the general form as closely as possible while removing all re-entrant wriggles.



**Fig. 3** Graphical representation of uncorrected (upper set) and corrected (lower set)  $^{14}\text{C}$  dates for the Ezero cultures<sup>15</sup> (see also Fig. 1). The open circles in the lower half of the figure represent fractional probabilities arising from wriggles in the calibration curve<sup>6</sup>. The median and quartile dates are shown by vertical bars.

season (50% of those from Ezero (Fig. 3) were seeds). Any reconstruction of the concentration of atmospheric  $^{14}\text{C}$  in the past which is to satisfy all the evidence must take archaeology into account, since it is not a discipline based entirely on value judgments. Excavations conducted with proper regard to stratification and to relations between finds are just as objective as records of the past as varves or tree rings. We hope that archaeologists will themselves work out the effects when all conventional  $^{14}\text{C}$  dates are corrected by any of the calibration curves now in use.

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## Cretaceous Foraminifers—Depth Habitats and their Origin

UPPER Cretaceous foraminifers, both planktonic and benthic, seem to have been depth distributed in a similar way to modern species. This hypothesis, examined primarily in terms of Upper Cretaceous foraminifera along the eastern North Pacific margin, explains the distribution patterns observed in Cretaceous shelf and slope foraminifers, the correlation of these faunas to modern assemblages, and the agreement of the patterns with the hydrographic conditions envisioned for Cretaceous oceans<sup>1</sup>. Here I show that these depth patterns originated in the mid-Cretaceous during the earliest of two periods of Cretaceous continental fragmentation and ocean floor spreading.

Upper Cretaceous planktonic foraminifers from Pacific shelf and slope habitats differ markedly in species composition and abundance (Fig. 1). Often species are more abundant in one depth habitat or the other or are restricted to a unique habitat<sup>2</sup>. Deep-water habitats are dominated by globotruncanids, with heterohellicids, hedbergellids and globigerinellids being less common. Conversely, shallow-water habitats are dominated by heterohellicids and globigerinellids with a marked increase in hedbergellids. A similar segregation of planktonic faunas has been observed in Cretaceous foraminifers from the western continental margin of the North Atlantic, the western interior of the United States<sup>3,4</sup>, the Gulf of Mexico<sup>5</sup>, and northern Europe<sup>6</sup>.

Upper Cretaceous benthic foraminifers also were depth distributed (Table 1). Parallel benthic communities<sup>7</sup> changed in species composition and thus succeeded one another along the bathymetric gradient of the continental margin<sup>8</sup>. The succession of genera is distinctly modern in appearance and contains several homeomorphs of modern bathymetric indicator species. So I make the assumption that the Cretaceous assemblages in this clastic environment occupied depth habitats similar to corresponding modern assemblages to depths of at least 2,000 m. According to this model, inner-shelf benthic faunas were dominated by miliolids, polymorphinids, encrusting genera and contained few agglutinated genera<sup>8</sup>. Outer-shelf assemblages were characterized by calcareous species chiefly nodosariids and the upper depth limits of deeper water genera such as *Hoeglundina*, *Gyroidina*, *Chilostomella* and *Glomospira*. Upper-slope environments were highly diverse and dominated by nodosariids and turrilids with increasingly abundant agglutinated genera. Middle-slope faunas were characterized by agglutinated species and representatives of the Turrilinae, Osangulariidae and Anomaliniidae whereas lower-slope faunas were dominated by agglutinated genera and deeper water calcareous species. A similar succession of Cretaceous foraminifers can be observed in assemblages from the western continental margin of the North Atlantic, the western interior of the United States<sup>9,10</sup>, the eastern Carpathians<sup>11</sup>, and Australia<sup>12</sup>.

Vertical distribution patterns of Cretaceous foraminifers are believed to be related directly to climatic and ocean current differentiation and the level of nutrient and food resources. Climatic variations<sup>13</sup> and ocean gyre configurations in the major ocean basins resulted in the establishment of planktonic provinces separated by thermal barriers. For example, planktonic foraminifers from the eastern North Pacific during the Upper Cretaceous (Campanian-Maastrichtian) define four faunal associations which can be referred to Boreal, Intermediate, Central and Tethyan faunal provinces<sup>2</sup>; the Intermediate and Central provinces in turn comprise a larger Transitional province. Within each province one would expect the plankton to respond to vertical thermal and resource gradients and become vertically segregated analogous to modern species<sup>14,15</sup>. This segregation was modified along the continental margin by the tolerance of certain species to shelf or environmentally variable planktonic habitats. So the bathypelagic chiefly globotruncanid assemblage was restricted

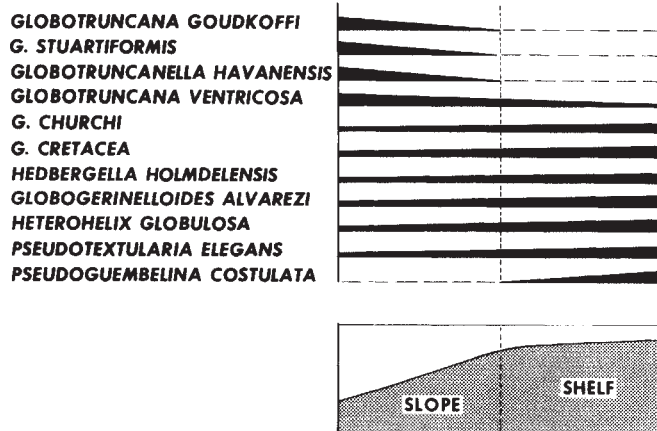


Fig. 1 Generalized depth distribution of Upper Cretaceous planktonic foraminifers from the eastern North Pacific margin. Height of bars proportional to species abundance. Schematic cross-section of depositional environments shown below.

to deeper water whereas the more rigorous shelf waters were occupied by opportunistic, largely epipelagic species characterized by heterohellicids and hedbergellids<sup>2</sup>.

The depth distribution of Cretaceous benthic foraminifers can be related to resource regimes within the hydrographic model. The progressive lowering of resource levels with

Table 1 Depth Assemblages of Upper Cretaceous Benthic Foraminiferal Genera from Mid-Latitude, Clastic Environments, data from ref. 8. Genera listed in decreasing order of ecologic significance from top to bottom and left to right in each bathymetric zone

| Environment |        | Foraminifers                                                                                                                                                                                                 |                                                                                                                                                                                                                     |
|-------------|--------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Shelf       | Inner  | Miliolids<br><i>Nonionella</i><br><i>Placopsilina</i><br><i>Guttulina</i><br><i>Globulina</i><br><i>Palmula</i><br><i>Epithemella</i>                                                                        | <i>Planorbulina</i><br><i>Vitriwebbina</i><br><i>Coryphostoma</i><br><i>Ceratobulimina</i><br><i>Bolivina</i><br><i>Pararotalia</i><br><i>Pseudopatulina</i>                                                        |
|             | Outer  | Nodosariids<br><i>Colomia</i><br><i>Hoeglundina</i><br><i>Alabamina</i><br><i>Gyroidina</i><br><i>Pleurostomella</i><br><i>Bolovina</i><br><i>Gavelinella</i><br><i>Fissurina</i>                            | <i>Seabrookia</i><br><i>Pyrulina</i><br><i>Pseudonodosoria</i><br><i>Gaudryina</i><br><i>Pseudouigerina</i><br><i>Dorothia</i><br><i>Coryphostoma</i><br><i>Pyramidina</i><br><i>Oolina</i>                         |
| Slope       | Upper  | <i>Osangularia</i><br><i>Gavelinella</i><br><i>Gyroidinoides</i><br><i>Hoeglundina</i><br><i>Silicosigmoilina</i><br><i>Bathysiphon</i><br><i>Gaudryina</i><br><i>Cribrostomoides</i><br><i>Praebulimina</i> | <i>Dorothia</i><br><i>Spiroplectammina</i><br><i>Ammodiscus</i><br><i>Trochammina</i><br><i>Colomia</i><br><i>Nodosariids</i><br><i>Tappanina</i><br><i>Pyramidina</i><br><i>Globulina</i>                          |
|             | Middle | <i>Praebulimina</i><br><i>Dorothia</i><br><i>Osangularia</i><br><i>Hoeglundina</i><br><i>Gaudryina</i><br><i>Chilostomella</i><br><i>Allomorphina</i><br><i>Pullenia</i><br><i>Planulina</i>                 | <i>Bathysiphon</i><br><i>Silicosigmoilina</i><br><i>Hyperammina</i><br><i>Cribrostomoides</i><br><i>Spiroplectammina</i><br><i>Gavelinella</i><br><i>Ammodiscus</i><br><i>Ammodiscoides</i><br><i>Stilostomella</i> |
|             | Lower  | <i>Glomospira</i><br><i>Bathysiphon</i><br><i>Hyperammina</i><br><i>Ammodiscus</i><br><i>Gaudryina</i><br><i>Cribrostomoides</i><br><i>Silicosigmoilina</i><br><i>Osangularia</i><br><i>Pullenia</i>         | <i>Praebulimina</i><br><i>Saccammina</i><br><i>Pelosina</i><br><i>Hormosina</i><br><i>Allomorphina</i><br><i>Gavelinella</i><br><i>Haplophragmoides</i><br><i>Spiroplectammina</i>                                  |

increasing depth and distance from shore resulted in a succession of increasingly stable and cosmopolitan populations<sup>16</sup> that apparently were confined to specific depth limits whereas their horizontal distribution was much greater similar to modern deep sea populations<sup>17,18</sup>. Further, the correspondence in depth distribution between Cretaceous and modern foraminifers suggests that the generic composition of foraminiferal assemblages can be predicted for different marine environments of this age. For example, high latitude faunas from the outer shelf and upper slope should contain a higher percentage of nodosariids and glandulinids than coeval lower latitude faunas; anaerobic middle-slope basins would be characterized by increased numbers of praebuliminids, alломorphinids and agglutinated genera when compared to adjacent open-slope faunas.

The modern generic appearance of the benthic assemblage seems to have originated in the mid-Cretaceous. Pre-Albian foraminiferal assemblages characteristically are cosmopolitan in nature. Deep sea benthic populations were dominated chiefly by nodosariids and a few agglutinated species while planktonic species belong to a few cosmopolitan genera<sup>19,20</sup>. The Cenomanian-Turonian saw a rapid turnover in marine and terrestrial biota second only to that at the culmination of the Mesozoic<sup>21</sup>. Foraminifers, too, underwent a period of extinction and appearance of many new genera resulting in rapid diversification of species during the Turonian and increased provincialism through the early Senonian<sup>22</sup>. The modern appearing benthic foraminiferal populations originated with this biotic turnover and the first occurrence of many of the modern benthic genera<sup>23</sup>.

Accompanying the biotic turnover were major changes in the geotectonic evolution of the ocean basins, sea level and climate. Concurrence of these events in the mid-Cretaceous and again in the late Cretaceous with the geotectonic evolution of the North and South Atlantic<sup>24,25</sup> seems more than coincidental and lends support to the hypothesis that continental fragmentation and ocean ridge development altered the volume of the ocean basins and produced changes in sea level, climate and resource levels and thus had a profound effect on the biota<sup>26</sup>. The pre-Albian North Atlantic resembled a closed equatorial basin with limited deep-water circulation as the rift system had not yet propagated into cooler latitudes. The Tethyal seaway extended along the equator and into the Pacific Ocean<sup>27</sup>, thus marine climates were equable with widespread tropics and subtropics<sup>28</sup>. These conditions were conducive to widespread marine populations with little geographic differentiation.

During the Aptian-Albian (100–112 m.y.) the rifting began in the late Jurassic (140 m.y.) separated Africa from India, Australia, Antarctica and South America<sup>29</sup>. Aptian salt deposits in Brazil and Africa record the first incursion of the sea leading to mixing of Tethyan and South Atlantic surface and intermediate water<sup>30</sup>. Deep circulation, however, was still blocked by the Rio Grande and Walvis ridges<sup>25</sup>. A widespread transgression occurred during the Albian-Cenomanian accompanied by major fluctuations in marine temperatures<sup>31</sup>.

The effects of these events on the biota were profound as circulation patterns were altered, climatic zonation was intensified and the epicontinental seas were flooded; all acted together to alter the environmental stability and nutrient supply. The net result was the selective extinction of many of the cosmopolitan, narrowly adapted species, followed by a burst of diversification and increased endemism as species occupied newly developed niches. Deep-water species accustomed to a highly stable, insulated environment and low resource levels particularly would be susceptible to changes in climate and ocean currents.

Conversely, the general generic composition of deep-water foraminiferal populations persisted during the biotic turnover at the end of the Mesozoic, whereas planktonic and shallow-water foraminifers were strongly affected<sup>23,32</sup>. It may be that the diversification and niche fractionation of the deep-

water benthic fauna following the early Senonian climatic deterioration pre-adapted the fauna to environmental changes at the end of the Mesozoic. Certainly, climatic fluctuations during the late Mesozoic<sup>13,31</sup>, opening of the Labrador Sea (80 m.y.)<sup>24</sup>, initiation of deep-water circulation in the South Atlantic (80 m.y.)<sup>25</sup> and continued latitudinal and longitudinal drifting of the continents would tend to reinforce the early Senonian deep-sea environmental conditions.

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## Surface Charge Characteristics of Hydroxyapatite and Fluorapatite

THE crystal and solution chemistry of apatites has been studied intensively by medical, biological and earth scientists but studies of the electrochemistry of apatite surfaces have not been extensive. The few studies of the surface charge characteristics of hydroxyapatite  $[\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6]$  and fluorapatite  $[\text{Ca}_{10}\text{F}_2(\text{PO}_4)_6]$  show that  $\text{H}^+$  and  $\text{OH}^-$  are potential determining ions, but estimates of the pH at the point of zero charge (PZC) have varied widely from 4.3 to 7.6 for hydroxyapatite<sup>1–5</sup> and from 4 to >12 for fluorapatite<sup>6–8</sup>.

This study was undertaken with particular concern for the stoichiometry purity of the solid, reduction of  $\text{CO}_2$  contamination and selection of indifferent supporting electrolyte. The measurement of charge utilized a titration technique<sup>9–16</sup>.



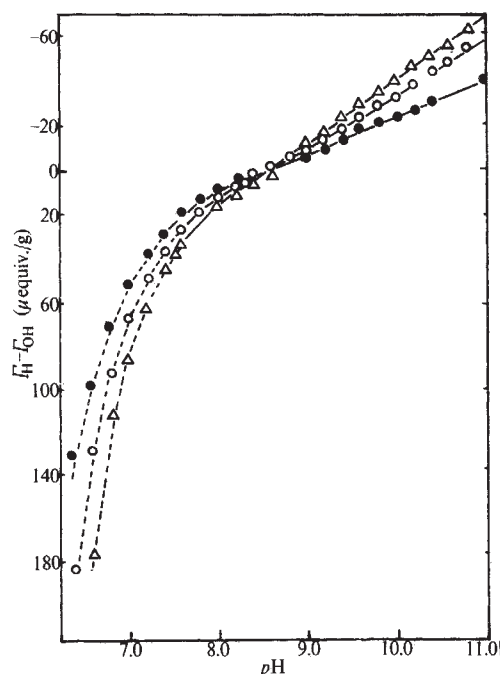


Fig. 1 Adsorption isotherms on hydroxyapatite B18 obtained by titration of suspensions (0.1 g/25 ml. KCl) after pre-equilibration for 16 h. Broken lines show where dissolution becomes significant.  $\Delta$ , 1 M;  $\circ$ , 0.1 M;  $\bullet$ , 0.01 M.

Synthetic hydroxyapatites of different surface areas were prepared by slowly adding equal volumes of  $\text{CO}_2$  free  $\text{Ca}(\text{OH})_2$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  solutions at a constant rate (100 to 1,000 ml./h) to rapidly stirred, distilled-deionized water at  $100^\circ\text{C}$  under  $\text{N}_2$  (pH 9.0). Equal volumes of each solution contained stoichiometric quantities of calcium and phosphorus.

Synthetic fluorapatite was prepared by the reaction of octacalcium phosphate  $[\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}]$  with  $\text{CO}_2$  free 0.1 M KF solution at  $40^\circ\text{C}$  for three weeks. Stoichiometric octacalcium phosphate was prepared by the slow hydrolysis of  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  in alkaline solution<sup>17</sup>.

The samples of apatites were well characterized by chemical, infrared, X-ray and electron diffraction analyses (Table 1), and were low in  $\text{CO}_3^{2-}$  which is known to substitute for  $\text{PO}_4^{3-}$  in apatite lattices<sup>18</sup>. The absence of any significant proportion (<2%) of acidic phosphate groups in the hydroxyapatite samples was indicated by infrared and X-ray diffraction analysis of a sample heated to  $1,000^\circ\text{C}$  for 6 days. No extraneous phases typical of reactions of acidic phosphate groups, such as  $\beta\text{-Ca}_3(\text{PO}_4)_2$  (ref. 19), could be detected.

Adsorption isotherms for  $(\Gamma_{\text{H}^+} - \Gamma_{\text{OH}^-})$  were measured on the apatite suspensions by automatic potentiometric titration in supporting electrolyte solutions of different ionic strengths (1.0, 0.1, 0.001 unless otherwise stated) at a rate of approximately 0.7 pH units  $\text{h}^{-1}$  (ref. 20), the technique being similar to that<sup>12</sup> used for iron oxides. The suspensions were equilibrated four times from 16 to 504 h before titration.

Isotherms were also constructed for some hydroxyapatite samples equilibrated for 144 h, by measuring the pH change in suspensions initially adjusted to varying pH values ("batch" procedure). The acid or alkali used as a titrant corresponded to the salt in which the solid was suspended.

Table 2 Effect of Solid to Solution Ratio, Electrolyte and Equilibration Time on the pH at the PZC of Hydroxyapatite and Fluorapatite

| Sample             | Solid/sol. g/25 ml. | Electrolyte                 | Equilibration time* h | pH at PZC     |
|--------------------|---------------------|-----------------------------|-----------------------|---------------|
| Hydroxyapatite B18 | 0.1                 | $\text{KNO}_3$              | 16                    | $8.5 \pm 0.1$ |
| Hydroxyapatite B18 | 0.1                 | $\text{KClO}_4$ †           | 16                    | $8.5 \pm 0.1$ |
| Hydroxyapatite B18 | 0.1                 | $(\text{CH}_3)_4\text{NCl}$ | 16                    | $8.4 \pm 0.2$ |
| Hydroxyapatite B18 | 0.1                 | $\text{NaCl}$               | 16                    | $7.6 \pm 0.1$ |
| Hydroxyapatite B18 | 0.1                 | $\text{KCl}$                | 16                    | $8.6 \pm 0.1$ |
| Hydroxyapatite B18 | 0.2                 | $\text{KCl}$                | 16                    | $8.6 \pm 0.1$ |
| Hydroxyapatite B18 | 0.1                 | $\text{KCl}$                | 144                   | $8.6 \pm 0.2$ |
| Hydroxyapatite B18 | 0.1                 | $\text{KCl}$                | 504                   | $8.5 \pm 0.2$ |
| Hydroxyapatite B34 | 0.1                 | $\text{KCl}$                | 16                    | $8.5 \pm 0.2$ |
| Hydroxyapatite B34 | 0.2                 | $\text{KCl}$                | 16                    | $8.3 \pm 0.2$ |
| Fluorapatite C34   | 0.1                 | $\text{KCl}$                | 16                    | $6.7 \pm 0.1$ |
| Fluorapatite C34   | 0.1                 | $\text{KClO}_4$ †           | 16                    | $6.8 \pm 0.2$ |
| Fluorapatite C34   | 0.1                 | $\text{KCl}$                | 504                   | $6.9 \pm 0.2$ |

\* Refers to equilibration time of suspension prior to titration except in the case of the 144 h treatment which involved equilibration of hydroxyapatite samples over a range of pH values for 144 h.

† 0.01 and 0.10 M only.

The PZC obtained from the cross-over point of the titration curves was approximately 8.5 (Table 2 and Fig. 1). This value was obtained irrespective of whether the supporting electrolyte was  $\text{KCl}$ ,  $\text{KNO}_3$ ,  $\text{KClO}_4$  or  $(\text{CH}_3)_4\text{NCl}$  providing evidence of the indifference of these electrolytes to the hydroxyapatite surface. The apparent indifference of  $\text{K}^+$  towards the hydroxyapatite surface is supported by recent work on the exchange of surface calcium on hydroxyapatite in the presence of  $\text{K}^+$  in solution<sup>21</sup>. In  $\text{NaCl}$  the cross-over point (pH 7.6) was significantly lower than that observed with indifferent electrolytes. It would appear that if the  $\text{Na}^+$  concentration in solution is high enough, exchange of surface  $\text{Ca}^{2+}$  by  $\text{Na}^+$  occurs, to produce a new surface of invariant composition having a lower PZC because of the increased negative charge. The size of the  $\text{Na}^+$  ion ( $r=0.95 \text{ \AA}$ ) is sufficiently close to that of the  $\text{Ca}^{2+}$  ion ( $r=0.98 \text{ \AA}$ ) so the exchange is not unexpected. Because of the interaction of  $\text{Na}^+$  with apatite surfaces, we suggest that it not be used as a supporting electrolyte in surface chemistry studies of these solids.

Varying the solid/solution ratio, surface area, and pre-equilibration ("ageing") period from 16 to 504 h did not alter the PZC of hydroxyapatite obtained in indifferent electrolytes (Table 2). The PZC did vary markedly with "ageing" time, however, if great care was not taken to exclude  $\text{CO}_2$ . Earlier reports of long periods to attain equilibrium are probably due to  $\text{CO}_2$  contamination.

No significant difference in PZC was observed between the automatic and batch titration procedures. There was, however, an increase in the  $\text{H}^+$  or  $\text{OH}^-$  adsorption on either side of the PZC with increasing time of equilibration by the batch pro-

Table 1 Some Characteristics of the Apatite Samples \*

| Sample               | Ca   | P    | Molar Ca/P† | F    | $\times 10^3$ |                    |    |    |     |     |     |     |    | Specific surface area $\text{m}^2/\text{g} (\text{N}_2)$ |
|----------------------|------|------|-------------|------|---------------|--------------------|----|----|-----|-----|-----|-----|----|----------------------------------------------------------|
|                      |      |      |             |      | Na            | $\text{CO}_3^{2-}$ | Cl | As | Mg  | K   | Fe  | Zn  | S  |                                                          |
| Hydroxyapatite (B34) | 39.4 | 18.2 | 1.67        | 0.02 | 3.7           | 60                 | 1  | 4  | 1   | 5.3 | 2.8 | 0.9 | 11 | 9.1                                                      |
| Hydroxyapatite (B18) | 38.8 | 18.1 | 1.66        | 0.02 | 19            | 40                 | 2  | <2 | 1.5 | 6.8 | 7.9 | 5.4 | 19 | 26.6                                                     |
| Fluorapatite (C34)   | 38.5 | 18.0 | 1.66        | 4.0  | 210           | 10                 | 6  | <3 | <1  | 98  | 21  | 4.0 | <2 | 35.3                                                     |

\* Analytical figures are given as percentages.

† Theoretical molar Ca/P of hydroxyapatite and fluorapatite is 1.66. Experimental values of the Ca/P listed in the table have an s.d. = 0.01.

cedure. This observation suggests that there is a slow diffusion of  $H^+$  into and out of the solid on the acid and alkaline side of the PZC respectively. At the PZC there is little tendency for  $H^+$  to diffuse into or out of the solid. Diffusion of  $H^+$  into the solid may be associated with the formation of a new phase—a more acidic phosphate. There is no more basic phosphate than hydroxyapatite known, and thus release of  $H^+$  on the alkaline side of the PZC cannot be attributed to the formation of a new phase.

The pH value at the PZC of hydroxyapatite in this study is from 1 to 4 pH units higher than that measured by other workers. The lower values obtained in these other studies could be attributed either to contamination of the hydroxyapatite with fluorapatite and/or proteins of lower PZC<sup>1,5</sup>, the use of NaCl as a supporting electrolyte<sup>3,4</sup> or the presence of  $CO_2$  and  $CO_3^{2-}$ , all leading to non-stoichiometry of the surface.

Fluorapatite was observed to have a PZC significantly lower than that observed for hydroxyapatite (Table 2). This value compares favourably with those found for a natural fluorapatite (PZC 6–7) by Somasundaran<sup>8</sup>. The lower PZC of fluorapatite is connected with the replacement of the structural OH groups parallel to the c-axis of the crystals by the fluorine atoms. The effect of this replacement could be two-fold: first, the substitution of a strong basic group ( $OH^-$ ) by a weakly basic group ( $F^-$ ); and second, the  $F^-$  atom, being more electronegative than  $OH^-$ , may decrease the basicity of nonstructural surface  $OH^-$  groups attached to the surrounding three coplanar calcium atoms<sup>22</sup> by drawing the shared electrons towards itself. This would in turn result in the shared electrons in any Ca—OH bonds being drawn closer to the calcium, thereby increasing the tendency for dissociation of  $H^+$  from these terminal groups.

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## Specification of Gradients used for Studies of Chemotaxis

THE responses of motile bacteria to various chemicals were studied in the 1880s by Pfeffer<sup>1</sup>, who inserted a capillary tube containing a solution of an attractant into a suspension of bacteria and noted that the bacteria accumulated first near the mouth of the capillary and then inside. The assay has been developed further by Adler<sup>2,3</sup>, who measured the number of bacteria which enter the capillary in a fixed period of time. With the advent of the tracking microscope<sup>4</sup> it has been possible to analyse responses of individual bacteria in detail<sup>5</sup>. For the analysis to be meaningful the concentration of the attractant must be known at each point in space and time. Therefore, we have solved the problem of diffusion from a small tube into a large pond. The solution also enables us to explain the general features of the Pfeffer assay.

A cylindrical capillary of radius  $r_c$  containing an attractant at concentration  $C_0$  is inserted at time  $t=0$  through the wall of a chamber containing a suspension of bacteria but no attractant (Fig. 1). The geometry is cylindrically symmetrical; therefore, a solution might be found by Wiener-Hopf<sup>6</sup> or two-dimensional numerical techniques. We have sought a simpler analytical solution. The capillary and the chamber may be considered semi-infinite provided that  $t < d^2/D$ , where  $d$  is the length of the capillary or the diameter of the chamber, whichever is smaller, and  $D$  is the diffusion constant of the attractant. Diffusion in the capillary is one-dimensional except near the mouth; diffusion in the chamber is (hemi-)spherical except near the mouth. Since a spherical field reduces to a one-dimensional one (dependent only on  $r$ ), an approximate solution can be obtained by joining two one-dimensional solutions<sup>7,8</sup>. This is done by forcing equality of the concentrations and fluxes at a small hemispherical surface of radius  $r_0 = \alpha r_c$  centred a distance  $l = \beta r_c$  from the wall of the chamber (Fig. 1). The dimensionless parameters  $\alpha$  and  $\beta$ , which are both of order unity, can be varied to fit experimental data. The solution in the pool is

$$C(r, t) = \frac{C_0 \gamma r_c}{r - r_0} \exp \left[ \frac{2\gamma(r - r_0)}{r_c} + \frac{\gamma^2 4Dt}{r_c^2} \right] \operatorname{erfc} \left( \frac{r - r_0}{2\sqrt{Dt}} + \frac{\gamma^2 \sqrt{Dt}}{r_c} \right) \quad (1)$$

where  $\gamma \equiv \alpha/(1 + 2\alpha^2)$ .

Once the diffusion has progressed farther than a few capillary radii ( $\sqrt{Dt} \gg r_c$ ) equation (1) is well approximated by

$$C(r, t) \simeq \frac{C_0 r_c^2}{2(r - r_0)(\pi Dt)^{1/2}} \exp \left[ \frac{-(r - r_0)^2}{4Dt} \right] \left[ 1 + \frac{r_c(r - r_0)}{\gamma 4Dt} \right] \quad (2)$$

and the total amount of attractant which has entered the pool is approximately

$$N(t) = C_0 2r_c^2 (\pi Dt)^{1/2} \quad (3)$$

A further useful approximation involves the choice  $r_1 \simeq r - r_0$  as the radius of the spherical coordinate system. If  $\alpha = \beta$ , the system is centred at the mouth of the capillary, as in Fig. 1. Choosing  $\alpha = 1$ , equation (2) becomes

$$C(r_1, t) \simeq \frac{C_0 r_c^2}{2r_1(\pi Dt)^{1/2}} \exp \left( \frac{-r_1^2}{4Dt} \right) \left( 1 + \frac{3r_c r_1}{4Dt} \right) \quad (4)$$

This equation can be integrated with respect to  $z$  (Fig. 1) in closed form, so it is possible to check the theory by loading



the capillary with a dye and measuring the amount of light absorbed from a ray normal to the axis of the capillary a distance  $L$  from its mouth (Fig. 2). A more refined comparison of the theory with experiment could be made with equation (1), but the integrations of  $C$  and  $C^2$  would have to be done numerically.

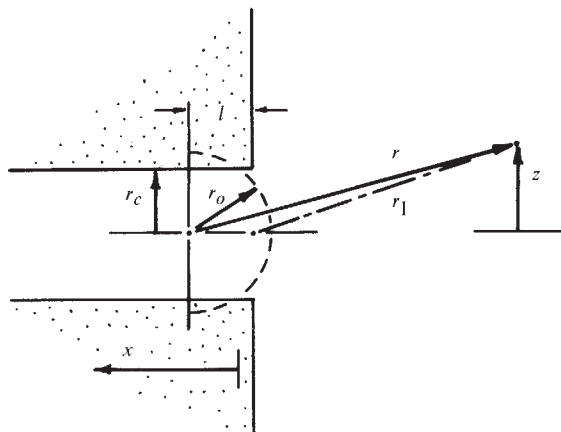


Fig. 1 The capillary (left) entering the pond (right).

In the capillary, with  $x$  measured from an origin near the mouth (Fig. 1), the solution is

$$C(x, t) = C_0 \operatorname{erf} \left( \frac{x}{2\sqrt{Dt}} \right) + \frac{C_0 \gamma}{\alpha} \exp \left( \frac{2\gamma x}{r_c} + \frac{\gamma^2 4Dt}{r_c^2} \right) \operatorname{erfc} \left( \frac{x}{2\sqrt{Dt}} + \frac{\gamma^2 \sqrt{Dt}}{r_c} \right) \quad (5)$$

which for  $\sqrt{Dt} \gg r_c$  simplifies to

$$C(x, t) \simeq C_0 \operatorname{erf} \left( \frac{x}{2\sqrt{Dt}} \right) + \frac{C_0 r_c}{\alpha(\pi Dt)^{1/2}} \exp \left( \frac{-x^2}{4Dt} \right) \left( 1 + \frac{r_c x}{\gamma 4Dt} \right) \quad (6)$$

An exacting analysis of the Pfeffer assay could be made from equations (1) and (5), but only a few of the essential features will be described here. When the capillary is inserted the concentration at the mouth falls to  $C_0/(1+2\alpha^2)$ , and a gradient develops nearby. The concentration elsewhere in the capillary is  $C_0$ ; the concentration elsewhere in the pond is zero. Only the bacteria in the immediate vicinity of the mouth respond. As the diffusion progresses the linear extents of the regions over which the concentration has changed and a gradient has appeared grow in the capillary and in the pond as  $\sqrt{2Dt}$ . The bacteria will follow these gradients as they form. A cloud of bacteria will develop in the pond, and bacteria will move into the capillary in a band. As the attractant spreads the gradients become weaker. If  $C_0$  is small, the response will be limited both in space and in time; the number of bacteria entering the capillary will be small. If  $C_0$  is much larger than the saturation value for the response, a region of saturation will expand out into the pond and then shrink again back into the capillary. If the saturation boundary fails to reach the capillary before the end of the experiment, the number of bacteria entering the capillary will again be small. If time is adequate and the gradient is not already too weak, bacteria will move into the capillary in a band in the wake of this boundary.

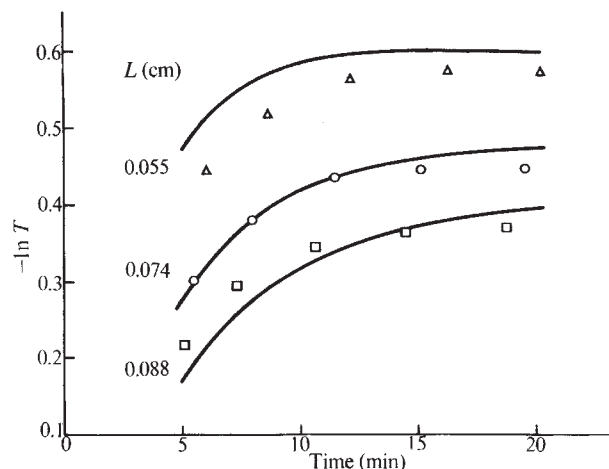


Fig. 2 An experimental test of equation (4).  $T$  is the transmittance, the ratio of the intensity of the ray in the presence of the dye to that in its absence. Solid lines: theory; symbols: experiment. A series of solutions of methylene blue were prepared in tracking medium<sup>5</sup> by dilution of a common stock solution (about  $5 \times 10^{-3}$  M). Their transmittances at 658 nm could be fitted accurately in the range  $10^{-5}$  M to  $10^{-4}$  M by an equation of the form  $-\ln T = (aC - bC^2)z$ , where  $a$  and  $b$  are constants and  $z$  is the thickness of the cell (2 mm). The  $C^2$  term arises because the dye dimerizes (half dimeric at about  $3 \times 10^{-5}$  M, refs. 9–11). Given this relation it was possible to measure the diffusion constant for mass transfer optically; we used a split rectangular cell of thickness 2 mm and monitored the movement of the dye from a solution at  $10^{-4}$  M to one at 0 M. Differences in the diffusion constant at the higher and lower concentrations were not evident in the data ( $D \approx 7.0 \times 10^{-6}$  cm<sup>2</sup>/s at 32° C). The capillary experiment was done in the tracker<sup>4</sup> in the chamber used for following the bacteria<sup>5</sup> ( $r_c = 0.01$  cm). The beam of light from the condenser of the microscope was narrow-banded (658 nm) and stopped down to 0.043 cm. Its intensity was measured immediately above the chamber with a fibre-optic pickup<sup>4</sup>. The theoretical values of  $-\ln T$  were computed by using equation (4) in the integration of  $(aC - bC^2)dz$  over the interval  $-\infty \leq z \leq \infty$ . Corrections for the finite size of the beam, albeit small, were made by averaging values of  $T$  computed for a series of points over its cross section.

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## BIOLOGICAL SCIENCES

Further Studies on Consecutive Matings in the *Anopheles gambiae* Complex

THE *Anopheles gambiae* complex consists of at least five closely related species; three of these pass their aquatic stages in fresh water and are provisionally designated *A. gambiae* A, *A. gambiae* B and *A. gambiae* C, and two are salt water breeders, *A. melas* and *A. merus*<sup>1</sup>. Crosses between any two of these species produce an F<sub>1</sub> generation in which the males are sterile. It has been suggested that release of these males be used to control the member species of this complex<sup>1,2</sup>. For such males to be effective in reducing a natural population it is essential that females which have mated with the sterile males do not subsequently become inseminated by normal males.

A preliminary field trial has been carried out using the F<sub>1</sub> males from the cross *A. gambiae* B male × *A. melas* female<sup>3</sup>. It has already been demonstrated that these hybrid males render their mates refractory to insemination by normal males<sup>4</sup>. Craig<sup>5</sup> has shown that in a number of species of mosquitoes monogamy is caused by matrone, a substance produced by the male accessory glands, which is transmitted to the female during copulation. It therefore seems unlikely that males with reduced accessory glands would make their mates monogamous. Such males would be of little value as control agents.

Crosses between *A. melas* males and species A females and *A. merus* males and species A females produce F<sub>1</sub> males with very reduced accessory glands<sup>1</sup>. Experiments were carried out to study the effects these males produced on their mates.

Mosquitoes used were virgin females and males of *A. gambiae* A from a colony established from material obtained in Pala (Upper Volta). Hybrid males were obtained from crosses between females of this colony and males of *A. melas* from a Gambian colony. Hybrid males from the cross between the Pala females and *A. merus* males from a colony from material collected near Tanga in Tanzania were also used.

Hybrid males were obtained from cage crosses. The parental females used in these crosses were isolated from the males within 18 h of emergence. As the male terminalia were not completely rotated by this time these females were assumed to be virgin. Virgin females for the mating studies were isolated in the same way.

Matings were carried out by forced or induced mating technique<sup>6</sup>. Each male was used to mate a maximum of two females to eliminate the possibility of depletion of accessory male gland substance through repeated matings.

One hundred virgin *A. gambiae* A females were mated with the F<sub>1</sub> males from the cross *A. melas* male × *A. gambiae* A female. A further 100 females were mated with these hybrid males and 24 h later were re-mated with *A. gambiae* A males. A similar series of experiments was carried out using the hybrid males from the cross *A. merus* male × *A. gambiae* A female. To determine the effects of mating on the females 100 virgin *A. gambiae* A females and 100 females which had been mated with homologous males were also studied.

The females were tubed individually, and fed blood as required. When they were gravid a small amount of water was added to the tubes for oviposition. The number of ovipositions and the number of batches of eggs which hatched were recorded.

The results are given in Table 1. Only two of the virgin females laid eggs whereas 32 of the homologously mated females oviposited. Oviposition in females which had been mated with either of the hybrid males was infrequent (4% and 5%). Doubly-mated females readily oviposited (63% and 46%) and the percentage of batches which hatched was high, showing that the second mating was the effective one.

These results show that the hybrid sterile males studied

here do not induce monogamy in their mates which can subsequently become inseminated by normal fertile males. Thus these males would be of little value as control agents against the member species of this complex.

Following the work of Craig<sup>5</sup>, the inability of these males to render females refractory to further copulation would seem to be due to impaired secretory activity of the accessory glands. The absence of accessory male gland substance in these males is also indicated by the behaviour of the females with which they have mated. Leahy and Craig<sup>7</sup> have demonstrated that in some species of mosquitoes, accessory male gland substance stimulates oviposition. The role of the accessory gland substance in inducing egg laying has been shown in the *A. gambiae* complex; virgin *A. gambiae* B seldom lay eggs<sup>1</sup> but do so readily after mating with the males from the cross *A. gambiae* B male × *A. melas* female<sup>4</sup>. These F<sub>1</sub> males have abnormal testes with incomplete spermatogenesis but well developed accessory glands. Hence the stimulation to oviposition occurs in the absence of spermatozoa. In this series of experiments, virgin *A. gambiae* A oviposited infrequently (2%) but those mated with homologous males often did so (32%). Females mated with F<sub>1</sub> males from the crosses between *A. gambiae* A females and the males of *A. melas* or *A. merus* behaved like virgin females with only 4% and 5% laying eggs.

**Table 1** Results of Mating *A. gambiae* Species A Females with Sterile and Fertile Males

| Males used for mating                                                                                     | No. of females | No. of ovipositions | No. of batches of eggs hatching | % of egg batches which hatched |
|-----------------------------------------------------------------------------------------------------------|----------------|---------------------|---------------------------------|--------------------------------|
| None (virgin females)                                                                                     | 100            | 2                   |                                 |                                |
| Species A♂                                                                                                | 100            | 32                  | 31                              | 96.9                           |
| F <sub>1</sub> ♂ of cross <i>A. melas</i> ♂ × species A♀                                                  | 100            | 5                   | 0                               |                                |
| First mating with F <sub>1</sub> ♂ of cross <i>A. melas</i> ♂ × species A♀, second mating with species A♂ | 100            | 63                  | 50                              | 79.4                           |
| F <sub>1</sub> ♂ of cross <i>A. merus</i> ♂ × species A♀                                                  | 100            | 4                   | 0                               |                                |
| First mating with F <sub>1</sub> ♂ of cross <i>A. merus</i> ♂ × species A♀, second mating with species A♂ | 100            | 46                  | 44                              | 95.7                           |

In *Aedes aegypti* the mechanism of action of matrone is behavioural<sup>8</sup>. The mated female holds her abdomen so that the male is unable to introduce the aedeagus into the vagina. In this species the forced copulation technique overcame such behavioural mechanism in 25% of the females but this does not seem to be the case in the *A. gambiae* complex. In an earlier series of experiments<sup>4</sup> it was found that *A. gambiae* B females which had mated with the F<sub>1</sub> males from the cross *A. gambiae* B male × *A. melas* female become monogamous and were refractory to insemination by fertile males even when the induced mating technique was used (the F<sub>1</sub> males from this cross have well developed accessory glands).

Competition experiments provide further evidence that the hybrid males used in these experiments do not render the females monogamous. When hybrid males were added to cages containing normal males and females of the *A. gambiae* complex the insemination rate of the females was similar to that of the control cages which contained only normal males and females<sup>9</sup>.

For sterile hybrid males to be of use as control agents they must have well developed and active accessory glands. Impairment of the secretory activity of the glands prevents the males from making their mates monogamous and so they can be re-mated and inseminated by normal, fertile males. Because



the  $F_1$  males from crosses between *A. melas* or *A. merus* males and *A. gambiae* A and *A. gambiae* B females have reduced accessory glands they cannot be used to control the member species of this complex.

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## Does X Chromosome Inactivation Occur during Mitosis of First Cleavage?

ACCORDING to the Lyon hypothesis the somatic cells of female mammals are functional mosaics with the X chromosome of maternal origin active in some cells and the paternal X active in the remainder<sup>1</sup>. Lyon also postulated that inactivation of X chromosomes occurs in early embryonic development and implied that, once inactivation occurs in any one cell, all descendants of that cell carry the same inactive X chromosome.

The developmental stage at which inactivation takes place has not been accurately determined, and one attractive possibility is that it occurs during the first cleavage mitosis in such a manner that the two blastomeres contain different active X chromosomes<sup>2</sup>. Equal numbers of cells from each of these two blastomeres should form the morula with considerable intermingling of those cells destined to be the inner cell mass. Mosaicism in accordance with the Lyon hypothesis should result if both clones are of equal vigour and are represented in the embryo proper so that they can contribute equally to the various tissue progenitors.

Tarkowski<sup>3</sup> demonstrated that it is possible to obtain viable offspring from single blastomeres of 2-cell embryos. If X inactivation occurs during the first mitosis, then females derived from such single blastomeres of embryos heterozygous for a sex-linked mutant marker gene should present phenotypes similar to animals homozygous for that mutant marker or for its wild-type allele. These two possibilities should occur with approximately equal frequency. Gartler and Nesbitt<sup>4</sup> attempted to test this hypothesis using the X-linked gene Tabby as the marker and X-rays to destroy blastomeres *in vivo*. We now report results using mechanical destruction of blastomeres *in vitro*.

Hybrid  $F_1$  female mice (C57BL/10Wt  $\times$  SJL/Wt) were mated to non-inbred  $Ta^1/Y$  males to produce embryos which were either  $Ta^1/(+)(\text{♀})$  or  $+/Y(\text{♂})$ . Two-cell embryos were flushed from the oviducts and immobilized by mild suction through a micropipette with an aperture of about 45  $\mu\text{m}$ . A glass probe of about 6  $\mu\text{m}$  diameter was manoeuvred with the aid of a Goldacre manipulator to puncture one of

the blastomeres and to disperse its cytoplasm<sup>5,6</sup>. The remaining blastomere, within the zona pellucida, was cultured for 48 h in a drop of medium under oil in an atmosphere of 5%  $\text{CO}_2$ , 5%  $\text{O}_2$ , and 90%  $\text{N}_2$ <sup>6,7</sup>. The cultured embryos (morulae or early blastulae) were transplanted to pseudopregnant females. Six to ten such half embryos were injected into each uterine horn and the foster mothers were allowed to deliver naturally (Mullen and Carter, unpublished work). The sex and phenotype of the young were recorded at about 4 weeks of age.

Two hundred and fifteen embryos developed in culture from 223 single blastomeres, of which 191 were transplanted into pseudopregnant females and 68 offspring were produced. Thirty-two of these were females and all were of the heterozygous phenotype. The 36 males were wild-type.

Dunn<sup>8</sup> recently observed that chimaeras produced by fusing embryos homozygous for the X linked gene Greasy with wild-type embryos produced animals whose phenotype resembled that of heterozygotes. It is unlikely that the animals we observed with the heterozygous phenotype were the result of chimaerism, because the embryos were transferred with the zonae intact and after the "sticky stage" at which chimaeras are easily produced. Also, from the results of Mullen and Whitten<sup>9</sup>, one would expect most of such chimaeras to appear as males whereas all 32 animals in this experiment with the heterozygous phenotype were females.

Our results indicate that X inactivation does not take place during the first cleavage. Gardner and Lyon<sup>10</sup> have recently obtained preliminary evidence that it may occur in the blastocyst. If this is correct then it would coincide with the first expression of paternal genes as established by Chapman *et al.*<sup>11</sup>.

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## Fusion of Frog and Tadpole Erythrocytes

THE development of cell fusion techniques has presented new possibilities in the study of nucleocytoplasmic relationships<sup>1</sup>. Where specific cell products can be traced, regulation of their synthesis by the heterokaryon nuclei can be elucidated. The switch from tadpole to adult frog haemoglobin in the bullfrog, *Rana catesbeiana*<sup>2,3</sup>, involves a change from synthesis of one predominant polypeptide subunit to another, or the inhibition of one structural gene and activation of the other (M. R., in preparation). I have fused tadpole and frog erythrocytes and followed the haemoglobin types synthesized by the heterokaryons.

Premetamorphic tadpoles were selected that showed a single anodal haemoglobin band on electrophoresis in acrylamide

gels<sup>4</sup>. Of the froglets which developed from tadpoles with a single anodal haemoglobin band, those froglets with a single cathodal haemoglobin band<sup>4</sup> were selected when the tail was 0.5 cm or less.

The tadpoles were anaesthetized in  $M_{222}$ , 1/2,000 (Sigma, St Louis, Mo.), dipped briefly in 70% ethanol, and bled into 10 ml. of sterile heparinized Ringer's solution<sup>5</sup> at 4° C by decapitation at the level of the truncus arteriosus. The froglets were pithed and bled by cardiac puncture with a 22 gauge needle and a heparinized 10 ml. syringe containing 10 ml. of Ringer's solution. The tadpole and frog blood samples were centrifuged at 500g for 5 min at 4° C. The plasma and white blood cell layer were removed, and 25  $\mu$ l. of the packed cells was resuspended in 10 ml. of sterile amphibian culture medium<sup>6</sup> made up without leucine and without serum. Centrifugation and resuspension in fresh medium were repeated twice. One ml. fractions of the suspensions were pipetted into sterile 60 mm 'Falcon' plastic Petri dishes to create a monolayer. When the cells settled to the bottom the medium was carefully removed with a Pasteur pipette, and the dishes were left at 7° C for 10 min.

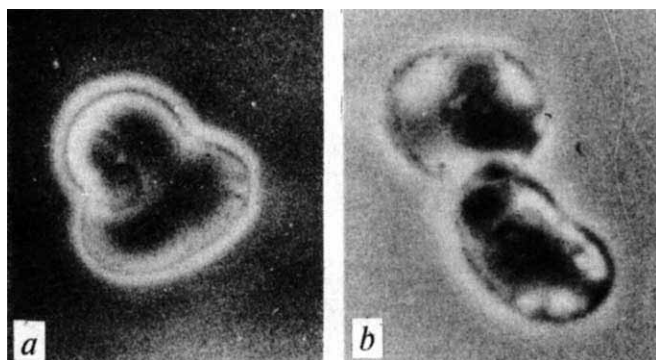


Fig. 1 Fusion of tadpole and frog erythrocytes. Tadpole erythrocytes plus Sendai virus were added to a dish of frog erythrocytes plus Sendai virus. *a*, RBC fusion 20 min after the addition of tadpole cells to frog cells; *b*, two dikaryons 3 h later.  $\times 650$ .

Very gently, 100 haemagglutinating units of Sendai virus<sup>7</sup> diluted to 1 ml. with amphibian culture medium (without leucine or serum) was added to the Petri dishes and these were left absolutely still at 7° C for 20 min. Two  $\mu$ l. of packed tadpole cells was added to 1 ml. of amphibian medium (without leucine or serum) containing 100 haemagglutinating units of Sendai virus. Two  $\mu$ l. of packed frog cells was similarly added to 1 ml. of medium plus virus. These samples were left on a gentle shaker at 7° C for 20 min.

After 20 min at 7° C the contents of the tubes were added, gently and drop by drop, to the Petri dishes, tadpole cells to frog cells, frog cells to tadpole cells, and controls of tadpole to tadpole and frog to frog. The dishes were left absolutely still at 7° C for 20 min.

Very gently, the dishes were removed to room temperature and the medium was removed with a Pasteur pipette. To each

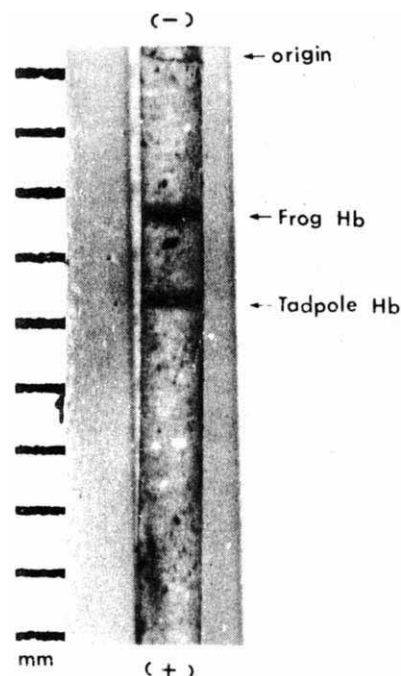


Fig. 2 Single cell electrophoresis<sup>4</sup> of a single dikaryon from the fusion of tadpole and frog erythrocytes. The gel was stained with the benzidine stain for haemoglobin<sup>4</sup>. Both tadpole and frog haemoglobins are present in the dikaryon. Fusion products of tadpole erythrocytes with tadpole erythrocytes and frog erythrocytes with frog erythrocytes revealed only one haemoglobin band each after single cell electrophoresis.

Petri dish was added 2.5 ml. of amphibian medium with serum and with 10  $\mu$ Ci of <sup>14</sup>C-leucine (specific activity = 250 mCi/mM; New England Nuclear Co., Boston). These dishes were left at 25° C for 3 h. At this stage complete fusion occurred (Figs. 1 and 2). Although there was about 20% lysis, the remaining erythrocytes were intact. Approximately 8% of the cells were dikaryotic products of fusion of two erythrocytes, 53% were single cells and the remainder were multikaryotic products of fusion of three or more erythrocytes.

The samples were centrifuged at 500g for 5 min. Obviously binucleate cells in the products were picked out by micromanipulation with a micropipette, lysed with water, and segregated into separate micro-capillary test tubes; this procedure has been published previously<sup>4</sup>. Electrophoresis of each dikaryon was performed on micro-acrylamide gels<sup>4</sup> for 5 min at 0.5 mA per tube. Of the gels containing the fusion products of tadpole with frog cells, 36 out of 50 revealed two haemoglobin bands after benzidine staining<sup>4</sup> (Fig. 2), approximately in the positions expected for tadpole and frog haemoglobins. None of the 50 gels containing the products of tadpole-tadpole and frog-frog fusion revealed more than one haemoglobin band, and 44 out of 50 of these gels revealed a single clear haemoglobin band. These results showed that the fusion of tadpole and frog erythrocytes was successful.

Autoradiography of the gels<sup>8</sup> revealed greater radioactivity over the frog haemoglobin bands than the tadpole haemoglobin

Table 1

| Experiment                                  | Frog-frog fusion |          | Tadpole-tadpole fusion |         | Frog-tadpole fusion |         | Tadpole-frog fusion |         |       |
|---------------------------------------------|------------------|----------|------------------------|---------|---------------------|---------|---------------------|---------|-------|
| Haemoglobin band                            | Frog             | Tadpole* | Frog*                  | Tadpole | Frog                | Tadpole | Frog                | Tadpole | Blank |
| <sup>14</sup> C counts per min in five gels | 315              | 18       | 13                     | 434     | 626                 | 208     | 729                 | 193     | 6     |
|                                             | 284              | 16       | 16                     | 397     | 718                 | 201     | 704                 | 211     | 5     |

\* These slices did not have haemoglobin on them but were taken as controls from the positions where haemoglobin bands would be expected.



bands in the gels from heterokaryons. The haemoglobin bands were sliced out from 25 gels containing tadpole-tadpole erythrocyte dikaryons, 25 gels containing frog-frog erythrocyte dikaryons, 25 containing tadpole-frog dikaryons, and 25 containing frog-tadpole dikaryons (tadpole-frog dikaryons are those in which a tube of tadpole cells was added to a dish of frog cells; frog-tadpole is the reverse). The slices were pooled in groups of five from each type and placed in glass scintillation vials (Packard, Downers Grove, Ill.). The slices were bleached and digested with 0.05 ml. of 7 g benzoyl peroxide in 30 ml. toluene plus 0.3 ml. NCS solubilizer (Amersham-Searle, Arlington Heights, Ill.) at 37° C overnight. Five ml. of scintillation mixture containing PPO, 15.2 g/gal., POPOP, 0.38 g/gal. (Amersham-Searle), and toluene to make one gallon, were added to each vial, and the vials were counted in a refrigerated three-channel Packard liquid scintillation spectrometer. Results are shown in Table 1.

In the heterodikaryons formed of tadpole and frog erythrocytes, tadpole haemoglobin synthesis was markedly reduced and frog haemoglobin synthesis was enhanced; the change was almost two-fold. The same result was recently obtained in cell-free haemoglobin synthesizing systems from *Rana catesbeiana* frog and tadpole erythrocytes: combination of lysates from frog cells with lysates from tadpole cells resulted in a dramatic increase in frog haemoglobin synthesis and reduction in tadpole haemoglobin synthesis<sup>3</sup>. It is reasonable to suppose that one or more genetic regulatory elements resides in these cells and controls the production of tadpole or frog haemoglobin. Biochemical fractionation might elucidate the nature of such genetic regulatory elements. Conceivably they are the same as those controlling the *in vivo* switch from tadpole to adult haemoglobin production in *Rana catesbeiana*.

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## Increased Plasma Enzyme Concentrations in Rats with Functional Ischaemia of Muscle provide a Possible Model of Duchenne Muscular Dystrophy

The early and mid-stage histopathological lesions of Duchenne muscular dystrophy are highly characteristic<sup>1</sup>. The early lesions are focal groups of muscle fibres undergoing necrosis surrounded by a field of normal muscle fibres, while in the

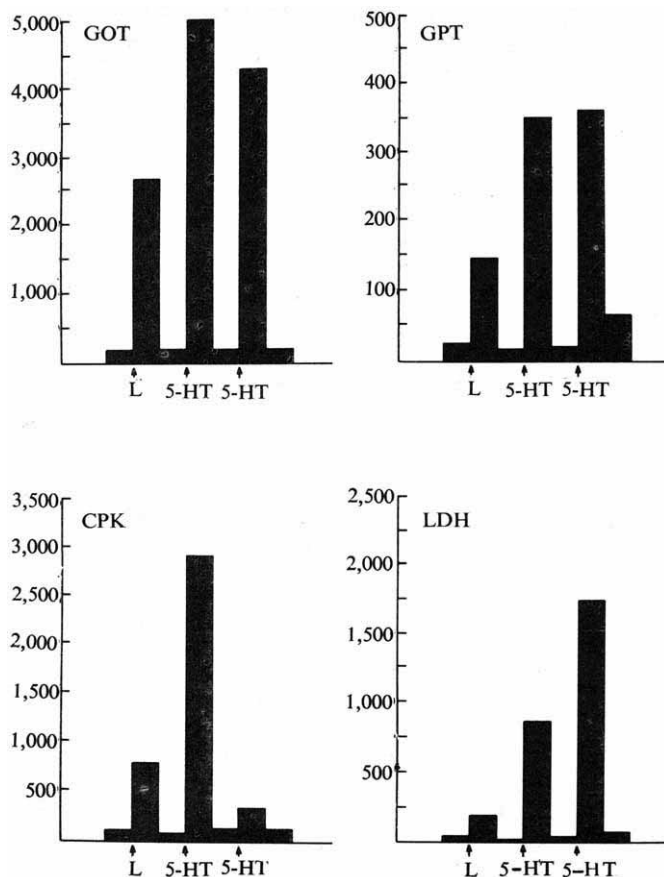


Fig. 1 Histograms of plasma enzyme levels in aorta-ligated rats showing values obtained for normal animals compared with peak increase and decrease following ligation (L) and two doses of 5-HT. Each vertical bar represents the mean enzyme value obtained from four animals. Units are explained in the text.

mid-stage lesions there is marked proliferation of endomysial connective tissue between muscle fibres that are small, enlarged and sometimes with internal nuclei. During these phases of the disease process, serum enzyme levels of creatine phosphokinase (CPK), glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT) and lactic dehydrogenase (LDH) are markedly increased<sup>2,3</sup>. These increases are often used in the diagnosis of Duchenne dystrophy.

We have demonstrated that functional ischaemia of the intramuscular blood vessels induced by a combination of aortic ligation and small doses of vasoactive agents, such as serotonin (5-hydroxytryptamine, 5-HT) or noradrenaline, can reproduce in rats the characteristic histopathological lesions of Duchenne muscular dystrophy<sup>4</sup>. (The phenomenon is termed "functional ischaemia" because of documented decreased blood flow following 5-HT without detectable histological changes in intramuscular blood vessels<sup>4</sup>.) For this animal model to be considered a close correlate of the human form of the disease, it should demonstrate similar serum enzyme increases. The study reported here has established that repeated functional ischaemia episodes to muscle in the rat are associated with increased enzyme levels, and also that the pattern of increases and decreases is somewhat similar to that of enzymes observed in patients with Duchenne muscular dystrophy. We measured CPK, GOT, GPT and LDH in plasma rather than serum because in rats enzymes are released from platelets during clotting, and this can artificially increase the results<sup>5</sup>.

103 male Osborne-Mendel rats (150–160 g) were used. All blood samples for enzyme determinations were obtained as follows. Under pentobarbital anaesthesia (35 mg/kg), 4 ml. of blood was removed by percutaneous left ventricular cardiac

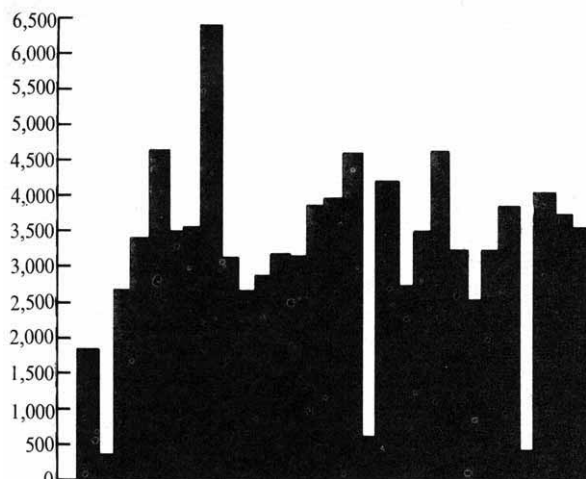


Fig. 2 Histogram of serum creatine phosphokinase levels determined at 2-3 day intervals during 3 months in an 8-yr-old boy with Duchenne muscular dystrophy.

puncture with a 25 gauge needle and a pre-heparinized syringe. The samples were immediately spun at 17,750g for 2 min in a refrigerated centrifuge. Plasma was separated and enzyme contents were determined by the following methods: GOT—Reitmann-Frankel method, expressed in IU<sup>6</sup>; GPT—Reitmann-Frankel method, expressed in U/ml.<sup>7</sup>; LDH—Babson-Phillips method, expressed in IU<sup>8</sup>; and CPK—Rosalki method, expressed in  $\mu$ U/ml.<sup>9</sup>

In normal rats anaesthetized as above, the abdominal aorta was ligatured below the renal arteries<sup>4</sup>. Plasma enzyme determinations were made at consecutive 24-h intervals after ligature. Intraperitoneal injections of 5-HT were given on the fifth and eighth days after ligature (20 and 25 mg/kg respectively). Blood samples were taken 12 and 24 h after each injection and then at consecutive 24-h intervals until enzyme concentrations returned to normal. Because obtaining samples for enzyme assay necessitated the death of the animal, serial blood samples could not be drawn from any single rat. Thus fifty-two rats were ligatured on day 1. Following ligature and subsequent injections of 5-HT, four rats were killed at each designated interval for plasma enzyme determinations. Each value (Table 1), therefore, represents the mean value obtained from four animals.

Thirty-one normal unligatured rats were used to establish normal plasma enzyme levels (Table 1). In a group of aorta-ligatured control rats injected with 1 ml. normal saline intraperitoneally on the fifth post-operative day, plasma enzyme levels determined at 12, 24, 48, and 72 h showed no increase.

The aorta-ligatured animals were fully recovered and clinically indistinguishable from normal rats (except for the operative scar) by 48 h. There was a marked increase of enzymes in all animals 24 h after ligature (Table 1). CPK and LDH levels had returned to normal by 72 h after ligature, and GPT and GOT were normal by 96 h. The maximum and subsequent minimum values of each enzyme after ligature and the two 5-HT injections are plotted serially in Fig. 1.

At the time of the first 5-HT injection (fifth post-operative day), the four plasma enzymes were within normal limits. They were markedly increased 12 h after injection of 5-HT (Table 1 and Fig. 1). LDH, GPT and CPK returned to normal by 48 h after injection, but GOT did not become normal until 72 h. By the time of the second 5-HT injection (eighth post-ligation day), the four plasma enzyme levels had returned to within normal limits. At 12 h after the second injection they were again markedly increased (Table 1 and Fig. 1). LDH and GPT levels returned to within normal limits within 48 h of the second injection, but CPK and GOT did not do so for 72 h.

These results demonstrate a considerable increase in plasma enzyme levels of CPK, GOT, GPT and LDH after the periods of 5-HT-induced functional ischaemia of rat skeletal muscle predisposed by aortic ligation. Foci of muscle necrosis have been correlated with this functional ischaemic episode<sup>4</sup>. The initial increase in plasma enzymes after aortic ligation alone probably resulted from a transient insult to the muscles of the lower extremities causing leakage of enzymes. It cannot be attributed to the operative procedure since plasma enzymes were normal after 24 h in sham-operated, unligatured control animals. Nor can it be attributed to widespread muscle necrosis, since morphological lesions after aortic ligation alone were not observed by Selye<sup>10</sup> and in our experience were limited to rare necrotic fibres and occasional internal nuclei<sup>4</sup>.

In ambulatory patients with Duchenne muscular dystrophy, serial basal serum enzyme determinations reveal a markedly fluctuating pattern<sup>11</sup>. This is illustrated by the basal serum CPK levels studied at 2-3 day intervals for 3 months in an 8-year-old Duchenne dystrophy patient (Fig. 2). All samples were obtained at 0700 h after the patient had remained in bed and fasted during the previous 10 h. The same type of pattern has been observed with LDH, SGOT and SGPT (unpublished

Table 1 Serial Plasma Enzyme Values obtained from Normal, Aorta-Ligatured Rats and Aorta-Ligatured Rats Injected with 5-HT

|                                                     | n  | GOT<br>Mean | s.d.  | n  | GPT<br>Mean | s.d. | n  | LDH<br>Mean | s.d. | n  | CPK<br>Mean | s.d. |
|-----------------------------------------------------|----|-------------|-------|----|-------------|------|----|-------------|------|----|-------------|------|
| Normal                                              | 31 | 100         | 17.2  | 31 | 33          | 9.8  | 31 | 35          | 15.1 | 31 | 63          | 19.4 |
| Ligatured (day 1)                                   |    |             |       |    |             |      |    |             |      |    |             |      |
| 24 h                                                | 4  | 2,589       | 1,762 | 4  | 146         | 84   | 4  | 219         | 170  | 4  | 810         | 684  |
| 48 "                                                | 4  | 704         | 559   | 4  | 62          | 24   | 4  | 70          | 43   | 4  | 145         | 92   |
| 72 "                                                | 4  | 552         | 156   | 4  | 63          | 43   | 4  | 53          | 11   | 4  | 65          | 36   |
| 96 "                                                | 4  | 137         | 92    | 4  | 27          | 5    | 4  | 22          | 9    | 4  | 59          | 20   |
| 120 "                                               | 4  | 102         | 21    | 4  | 27          | 5    | 4  | 22          | 13   | 4  | 47          | 16   |
| First 5-HT injection<br>(fifth post-ligation day)   |    |             |       |    |             |      |    |             |      |    |             |      |
| 12 h                                                | 4  | 5,200       | 1,780 | 4  | 352         | 126  | 4  | 901         | 105  | 4  | 2,925       | 681  |
| 24 "                                                | 4  | 447         | 392   | 4  | 56          | 12   | 4  | 66          | 21   | 4  | 211         | 26   |
| 48 "                                                | 4  | 260         | 281   | 4  | 39          | 15   | 4  | 35          | 13   | 4  | 62          | 41   |
| 72 "                                                | 4  | 125         | 18    | 4  | 30          | 8    | 4  | 32          | 9    | 4  | 67          | 28   |
| Second 5-HT injection<br>(eighth post-ligation day) |    |             |       |    |             |      |    |             |      |    |             |      |
| 12 h                                                | 4  | 4,240       | 535   | 4  | 364         | 130  | 4  | 1,745       | 464  | 4  | 450         | 271  |
| 24 "                                                | 4  | 3,362       | 892   | 4  | 249         | 34   | 4  | 2,169       | 70   | 4  | 262         | 241  |
| 48 "                                                | 4  | 231         | 63    | 4  | 35          | 6    | 4  | 41          | 11   | 4  | 172         | 57   |
| 72 "                                                | 4  | 145         | 97    | 4  | 62          | 15   | 4  | 79          | 40   | 4  | 101         | 17   |



results of J. R. M. and W. K. E.). Our animal model suggests that in Duchenne dystrophy this fluctuating serum enzyme pattern represents repeated small episodes of functional ischaemia, resulting in focal areas of muscle necrosis accompanied by brisk elevations of serum enzyme levels followed by decreases in the next 48–72 h. During the early years of the disease a gradual increase of baseline enzyme levels would result from the accumulative muscle destruction present at any one time, with superimposed peaks possibly corresponding to acute episodes of functional ischaemia.

The importance of this study is the demonstration that the induced experimental animal model of Duchenne muscular dystrophy consisting of aortic ligation plus vasoactive amines, in addition to reproducing the highly characteristic early and mid-stage histological lesions of the clinical disease<sup>4</sup>, correlates in a second major aspect, namely, marked elevations and falls of enzyme levels of CPK, GOT, GPT and LDH, giving a pattern somewhat similar to that of Duchenne dystrophy patients. This animal model not only provides an opportunity to study the pathogenesis of a functional ischaemic myopathy but also adds the potential for screening drugs which might be therapeutically useful in human functional ischaemic myopathy, which is the pathogenesis we propose for Duchenne muscular dystrophy.

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## Oral Transmission of *Brugia pahangi* to Jirds (*Meriones unguiculatus*)

WHEN Manson first incriminated mosquitoes in the transmission of *Wuchereria bancrofti*<sup>1,2</sup>, he suggested that filariasis is acquired by drinking water in which infected mosquitoes had died. Later evidence showed that mosquitoes could convey infective larvae directly to human skin<sup>4</sup>. Yokogawa later revived the issue of oral transmission but concluded that infection *per os* was highly improbable<sup>5</sup>. It seems, therefore, that no one has hitherto demonstrated transmission of *W. bancrofti* or any other mosquito-"nursed" filarial worm by mouth. We report here oral infection of the dark-clawed Mongolian jird (gerbil), *Meriones unguiculatus*, with the filarial worm *Brugia pahangi*.

Third-stage larvae of *B. pahangi*, dissected from *Aedes aegypti* (Liverpool strain<sup>6</sup>) that had fed on a microfilaraemic jird 15 days earlier, were collected in an insect Ringer's solution<sup>7</sup> and pipetted into the mouths of 4 male and 3 female jirds

anaesthetized with Nembutal; each jird received about 100 worms in less than 0.3 ml. of the fluid. For comparison, 2 male jirds and 1 female each received, under anaesthetic, 100 third-stage larvae subcutaneously in the groin<sup>8</sup>. Starting 9 to 10 weeks post-exposure, and weekly thereafter, we examined 0.02 ml. of retro-orbital blood from each jird for microfilariae. At about 21 weeks post-exposure we killed the jirds and searched for adult worms in the carcasses whose dismembered parts were soaked separately in phosphate-buffered saline (pH 7.2) for 4 h at room temperature.

In those animals orally infected microfilariae appeared in the orbital blood in 3 of the 4 male jirds and in 1 of the 3 females about 10 to 15 weeks post-infection. In both of the microfilariae-"negative" females, but not in the "negative" male, microfilariae were later found in the heart blood at necropsy. The number of circulating microfilariae per 0.02 ml. of blood varied but usually did not exceed about 10. Each of the 7 jirds contained adult parasites at necropsy; we recovered a total of 52 worms (7.4% of those administered), all but 2 from the heart-lungs or chest cavity, and none from the gonads.

In animals infected subcutaneously, after the nominal prepatent period, microfilariae appeared in the orbital blood of the female jird and in one of the two males; heart blood from the microfilariae-"negative" male contained no microfilariae at necropsy. Circulating microfilariae in the patent female reached 14 per 0.02 ml. of orbital blood, and in the patent male 330. Each of the 3 jirds yielded adult worms at necropsy; we recovered thirty worms in all (10% of those administered); ten from the heart-lungs and chest cavity, and most of the rest from the gonads and abdominal viscera.

Infection with *B. pahangi* became established *per os* in jirds of both sexes; microfilariae circulated in many of the animals, and about the same proportion of worms matured after oral as after subcutaneous infection. (A second experiment confirmed these findings.) Our data indicate, furthermore, that over 90% of the adult worms developing from oral infections localized in the heart-lungs or chest cavity, whereas those developing from subcutaneous infections were distributed in the heart-lungs, chest cavity, gonads, abdominal viscera, or elsewhere<sup>8</sup>. Ash and Riley<sup>8</sup> suggest that the presence of adult *B. pahangi* in the heart-lungs of jirds indicates a poor host-parasite relationship because these filariae parasitize only lymphatic trunks in natural hosts. Nevertheless, because worms matured and microfilariae circulated in our orally infected jirds, the *B. pahangi*-jird model gives a tool for continued study.

Definitive hosts other than jirds may be susceptible to oral infection with *B. pahangi*, worms of related filarial species may also be transmissible orally, and cryptic infection by mouth may perhaps prove important in the genesis and epidemiology of human filariasis and tropical pulmonary eosinophilia. These speculations do not affect the accepted concept of vector-to-skin transmission of filariasis, but add an as yet new component to the epidemiology of filariasis.

In other experiments we have found that infective larvae of *B. pahangi* migrate spontaneously from dead or dying mosquitoes kept on water, and that many of the larvae remaining in such mosquitoes migrate to the abdomen and remain alive for several days. These observations lend substance to Manson's speculation that larvae escape from "... the dying mosquito, and finally obtain liberty in the water into which the insect has fallen"<sup>3</sup>.

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## Flying Ability of *Archaeopteryx*

YALDEN, in calculating the flying speed of *Archaeopteryx*, states<sup>1</sup> that he includes the areas of the body strip and tail, and assumes the same lift coefficient for both the wing and tail surfaces. Heptonstall in an earlier letter<sup>2</sup> does not include the body strip because "it does not behave like an aerofoil in generating high lift".

The accepted definition of the lift coefficient and wing area, given by Etkin<sup>3</sup> and others<sup>4</sup>, is

$$C_L = C_{L(\text{wing})} + C_{L(\text{tail})}, A_{\text{tail}}/A_{\text{wing}}$$

where  $C_{L(\text{wing})}$  refers to the wing plus body combination only, and  $A_{\text{wing}}$  includes the body strip but excludes the tail area, by definition.  $C_{L(\text{tail})}$  may be either positive or negative, depending on an analysis of the tail angle required to balance the pitching moment from the wing-body combination<sup>3,4</sup>.

Inclusion of the body strip in the wing area is important for two reasons. First, wing lift depends on the total (wing span)<sup>2</sup> according to Glauert<sup>5</sup> and others<sup>6,7</sup>, and, second, the presence of a body increases the lift of an isolated wing through mutual interference effects which become significant at low aspect ratios as shown, for example, by Flax and Lawrence<sup>7</sup>.

Good design practice requires that the tail should stall after the wing so that the tail operates at a lower lift coefficient than the wing, in general, but it is doubtful if the refinement which results from including the tail lift is justified by the accuracy of the data available, or the difficulty in estimating  $C_{L(\text{tail})}$ . The error in flying speed as a result of neglecting the tail lift is certainly no greater than that arising from an incorrect definition of wing area.

My own estimate of the minimum flying speed of *Archaeopteryx*, based on a wing lift coefficient of 1.3 but neglecting tail lift, varies from 7 m s<sup>-1</sup> at a wing loading of 0.4 g cm<sup>2</sup> to 11 m s<sup>-1</sup> at a wing loading of 1.0 g cm<sup>2</sup>.

Bramwell claims<sup>8</sup> that the tail lift of *Archaeopteryx* cannot be ignored because it contributes a 20% reduction in stalling speed. If this were true, it can be shown that the tail lift must support 36% of the total weight and is equal to 56% of the wing lift. Because the tail area is only 29% of the wing area<sup>8</sup>, it follows that the tail must operate at nearly twice the lift coefficient of the wing, and must have evolved with significantly better high lift characteristics.

There is another objection to the importance which Bramwell attaches to the tail lift. If the tail contributes a significant proportion of the total lift, then even a small deflexion of the tail surface for longitudinal control purposes will alter the balance of lift and weight forces, and result in an oscillatory motion which will be difficult to control. *Archaeopteryx* would be a difficult machine to fly. In practice, the tail lift force is designed to be as small as possible in order to minimize the total lift induced drag, the weight of tail structure, and changes in total lift resulting from one of its primary functions as a control surface.

The other essential function of the tail surface is to provide positive stability, as was pointed out by both Bramwell and Heptonstall. If, as they suggest, the tail does contribute some positive lift, then, from balance considerations, the centre of gravity must be located behind the aerodynamic centre of the wing-body combination (excluding the tail). This is a basically unstable configuration. Furthermore, the low aspect ratio of

the tail indicates that it is aerodynamically less effective (that is, it has a lower lift gradient) than a broad tail configuration.

A large tail area will be required simply to shift the aerodynamic centre to a stable position behind the centre of gravity, and also to provide effective pitch control at low flying speed. Many aircraft have a large tail surface for this reason.

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## Experiments on Mimicry: Gestalt Perception and the Evolution of Genetic Linkage

POLYMORPHIC mimicry in butterflies requires that their potential predators, either birds, lizards or monkeys, should be able to exercise Gestalt perception. We here describe a simple experiment showing that wild birds are indeed able to perceive a pattern as a whole, rather than responding only to single elements of it.

Accurate batesian mimicry (the close resemblance of a palatable insect to an unpalatable one) can evolve gradually rather than by a single step, for even poor mimics are to some extent protected from predators<sup>1,2</sup>. Mimicry probably did evolve in this way, in lepidoptera, for geographical races which mimic different models are usually differentiated by several genetic loci in different linkage groups; this is true of the batesian mimic *Papilio dardanus*<sup>3</sup> and of the muellerian mimics *Zygaena ephialtes*<sup>4</sup> and *Heliconius melpomene*<sup>5</sup>. But in breeding experiments with a polymorphic mimetic species, however, several forms which occur in the same population differ by major alleles at a single genetic locus even though their patterns may have few features in common (as in the batesian mimics *Papilio memnon*<sup>6</sup> and *P. dardanus*<sup>7</sup>).

Clarke and Sheppard<sup>8</sup> have explained this anomaly by suggesting that the apparently single mutations are in fact clusters of closely linked loci, and that the gradual evolution of mimicry has been accompanied by the evolution of close linkage, because natural selection acts against recombinant genotypes. If, for example, *Papilio memnon* had forms mimicking two model species, *A* and *B*, with differently coloured bodies and differently patterned wings, a form of *P. memnon* having the *A* body with the *B* wings, or vice versa, would resemble neither model, and would be at a selective disadvantage, so that selection would act to increase the linkage between genes controlling body colour and wing pattern<sup>9</sup>.

Only predators who perceive the butterfly as a whole will produce this disruptive selection, for predators, noticing only the colour of the body or only the wing-pattern, will avoid all four kinds of mimic, without especially eliminating those with the wrong combinations of bodies and wings. Clarke and Sheppard<sup>10</sup> give biometrical evidence that such Gestalt perception is affecting the variance of tail-length in the Abyssinian race of *Papilio dardanus*, but there is no direct evidence of this behaviour by insectivorous birds.



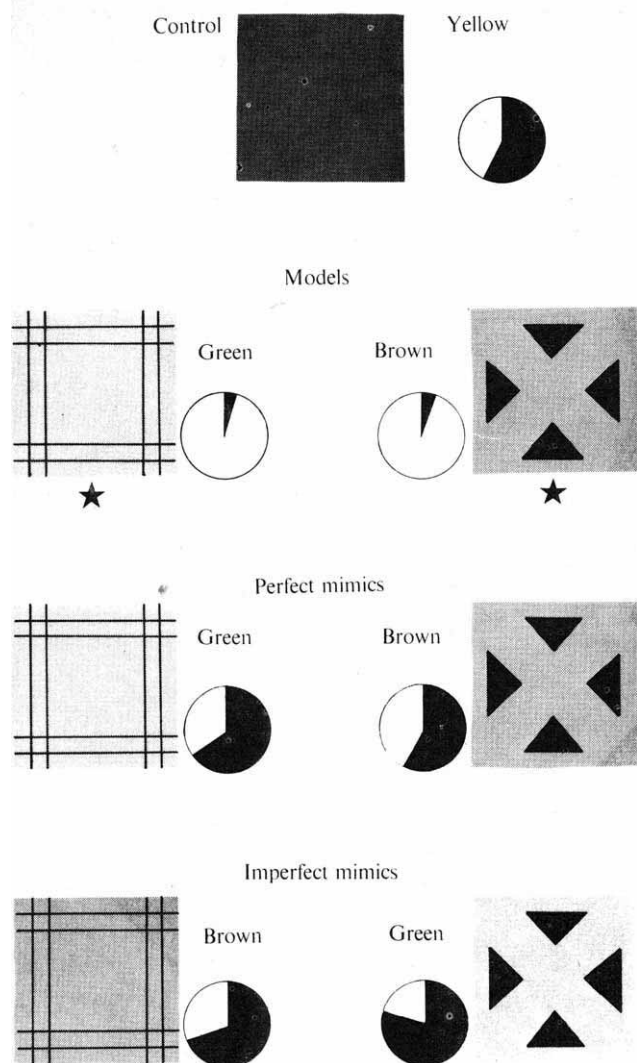


Fig. 1 The seven types of card used in the experiments; a star indicates that the pastry (not shown) was unpalatable. Black segment of pie is percentage eaten in experiment 1. Colours were Rowney waterproof inks: middle, green, burnt Sienna and yellow 02; the black patterns were in black waterproof ink.

We therefore carried out three experiments using artificial prey fed to wild birds, following a technique described in earlier papers<sup>2,11,12</sup>. Pieces of fresh pastry were placed on squares of coloured cardboard (5 × 5 cm) in a randomized arrangement on a suburban lawn in Crewe. The chief predators were sparrows (*Passer domesticus*) and starlings (*Sturnus vulgaris*), with sporadic visits from blackbirds and song thrushes (*Turdus merula* and *T. philomelos*). Seven kinds of prey were used (Fig. 1): a plain yellow control, two models (green with a pattern of black lines and brown with a pattern of black triangles), and four mimics, two exactly like the models and two with the reverse combinations of colour and pattern. The pastry on the model cards (distinguished by a cross underneath, invisible to the birds) was made unpalatable by soaking in a 70% solution of quinine dihydrochloride for 45 min; the pastry on the other cards was soaked in tap water. Trials, one each day, beginning at 0630 h BST in the summer and 0900 h BST in the winter, were continued until 10 of the 16 control prey had been eaten, as near as could be judged by watching the birds from a nearby window. Prey were scored as "eaten" or "not eaten". Each experiment was preceded by a fortnight of preconditioning, in which the birds were first trained to visit the lawn at the right time by being fed on scraps, and then were trained to eat pastry from cards, the yellow control cards only being presented.

Table 1 Details of the Three Experiments

| Experiment | Dates                | Number of prey tested in each trial |          |          |                    |
|------------|----------------------|-------------------------------------|----------|----------|--------------------|
|            |                      | Control                             | GL model | BT model | Each type of mimic |
| 1          | Aug 6–Sep 2, 1970    | 16                                  | 16       | 16       | 8                  |
| 2          | Dec 20–Jan 9, 1970–1 | 16                                  | 26       | 6        | 8                  |
| 3          | Aug 1–Aug 28, 1971   | 16                                  | 16       | 16       | 8                  |

| Mean No. of prey eaten per trial |         |      |      |        |      |      |      |               |
|----------------------------------|---------|------|------|--------|------|------|------|---------------|
| Experiment                       | Models  |      |      | Mimics |      |      |      | No. of trials |
|                                  | Control | GL   | BT   | GL     | BL   | GT   | BT   |               |
| 1                                | 10.07   | 0.79 | 0.64 | 5.46   | 5.86 | 6.36 | 5.00 | 28            |
| 2                                | 9.10    | 1.67 | 0.33 | 5.95   | 5.33 | 6.29 | 4.95 | 21            |
| 3                                | 9.79    | 0.82 | 0.39 | 5.75   | 5.61 | 6.39 | 4.75 | 28            |

G = green, B = brown, L = lines, T = triangles.

Mean numbers of prey eaten in each experiment and the numbers used in each trial are given in Table 1; Fig. 1 shows the percentages eaten in the first experiment. With equal numbers of both kinds of model (as in experiments 1 and 3) we expect neither colour (green or brown) nor pattern (lines or triangles) to have any influence on the predation mimics; and the analysis of variance on experiment 1 confirms this (Table 2). If the predators are unable to perceive the colour and pattern of the models as a whole, then the perfect mimics should be no better protected than the other mimics with the colour and pattern reversed. On the other hand, if some of the predators exercise

Table 2 Analysis of Variance on the Numbers of the Four Types of Mimic Eaten in the Three Experiments

| Factor             | Sum of squares | df  | Mean square | F    | P       |
|--------------------|----------------|-----|-------------|------|---------|
| Experiment 1       |                |     |             |      |         |
| Colour             | 6.51           | 1   | 6.51        | 1.73 | 0.19    |
| Pattern            | 0.01           | 1   | 0.01        | 0.00 | No test |
| Time (days)†       | 202.03         | 27  | 7.48        | 3.38 | 0.0012  |
| Colour × pattern † | 21.44          | 1   | 21.44       | 9.68 | 0.0044  |
| Colour × time      | 42.74          | 27  | 3.80        | 0.71 | No test |
| Pattern × time     | 18.24          | 27  | 2.89        | 0.31 | No test |
| Deviation          | 59.81          | 27  | 2.22        | —    | —       |
| Total              | 350.78         | 111 | —           | —    | —       |
| Experiment 2       |                |     |             |      |         |
| Colour*            | 20.01          | 1   | 20.01       | 5.90 | 0.020   |
| Pattern            | 0.01           | 1   | 0.01        | 0.00 | No test |
| Time (days)        | 67.81          | 20  | 3.39        | 1.71 | 0.12    |
| Colour × pattern   | 2.68           | 1   | 2.68        | 1.35 | 0.26    |
| Colour × time      | 28.24          | 20  | 1.41        | 0.71 | No test |
| Pattern × time     | 37.24          | 20  | 1.86        | 0.94 | No test |
| Deviation          | 39.57          | 20  | 1.98        | —    | —       |
| Total              | 195.56         | 83  | —           | —    | —       |
| Experiment 3       |                |     |             |      |         |
| Colour*            | 22.32          | 1   | 22.32       | 4.64 | 0.036   |
| Pattern            | 0.32           | 1   | 0.32        | 0.07 | No test |
| Time (days)        | 104.25         | 27  | 3.86        | 1.79 | 0.068   |
| Colour × pattern*  | 15.75          | 1   | 15.75       | 7.30 | 0.012   |
| Colour × time      | 71.68          | 27  | 2.65        | 1.23 | 0.30    |
| Pattern × time     | 57.68          | 27  | 2.13        | 0.99 | No test |
| Deviation          | 58.25          | 27  | 2.16        | —    | —       |
| Total              | 330.25         | 111 | —           | —    | —       |

\* Significant 5%.

† Significant 1%.

F ratios for time, and for all the interactions are obtained by using the deviation mean square; F for colour and pattern is obtained by using the deviation mean square plus the appropriate interaction mean square<sup>15</sup>.

*Gestalt* perception, the perfect mimics should be better protected, and this will appear as a significant colour  $\times$  pattern interaction. Table 2 shows that in experiment 1 the interaction of pattern with colour is highly significant, and we must conclude that at least some of the birds are capable of this simple kind of *Gestalt* learning.

Experiment 3, a replication of experiment 1 but performed a year later, confirms this result (Table 2): the interaction of colour and pattern is again significant. In this experiment, however, colour is also significant, green prey being eaten more than brown. This unexpected result does not contradict our predictions, because more green unpalatable models are eaten than brown (Table 1). For some reason the birds prefer to feed from the green cards, whether models or mimics. This may be an innate preference, or because of some further associations which the colours have for them. Birds may prefer one colour of model to another, probably as a result of the control acting as a poor batesian mimic of the preferred model<sup>12</sup>.

Experiment 2, which differs from the others in being performed in winter and in having the green models outnumbering the brown 26:6 (Table 1), gives an anomalous result. As the mean square colour  $\times$  pattern interaction is not significantly greater than its error (Table 2), the experiment fails to demonstrate *Gestalt* learning. The significant results, however, of experiments 1 and 3 suggest that *Gestalt* perception is not generally absent, but that there are circumstances in which it may disappear or become insignificant. In some experiments, the frequency-dependence of selection on a polymorphic batesian mimic can also disappear<sup>13</sup>. The protection afforded by mimicry in all three of our experiments was marginal; the birds ate very few of the unpalatable models, but ate roughly the same proportion of mimics as palatable controls. In similar experiments, birds have distinguished models from "perfect" mimics, possibly because the quinine causes a slight yellowing of the pastry<sup>2,12</sup>. In this series any pastry pecked but not consumed was recorded as "not eaten", so some of the models may have been tried and rejected. However, there is no reason to doubt that the colour  $\times$  pattern interaction of experiments 1 and 2 is due to mimicry, as batesian mimicry has previously been very effectively demonstrated<sup>2,13,14</sup>.

*Gestalt* perception of models and mimics may perhaps be exercised by only some predators some of the time. The evidence of these experiments, showing that it is exercised by unconfined wild birds when feeding in a more or less natural way, and the biometrical evidence for its action on natural populations of *Papilio dardanus*<sup>10</sup>, strongly support the contention that *Gestalt* learning is responsible for the tight genetic linkage found in polymorphic batesian mimics.

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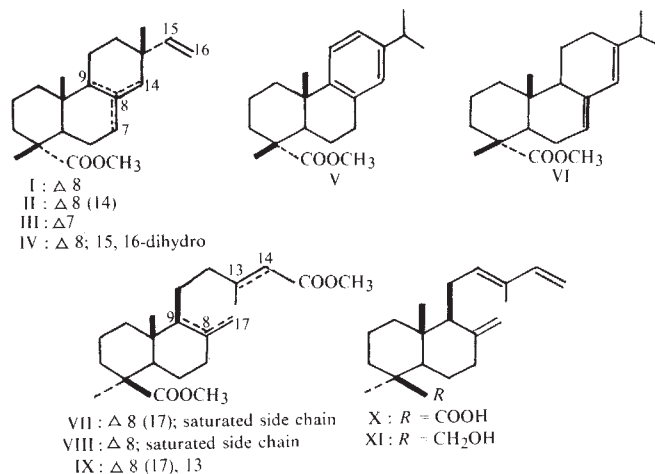
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## The Composition of Succinite (Baltic Amber)

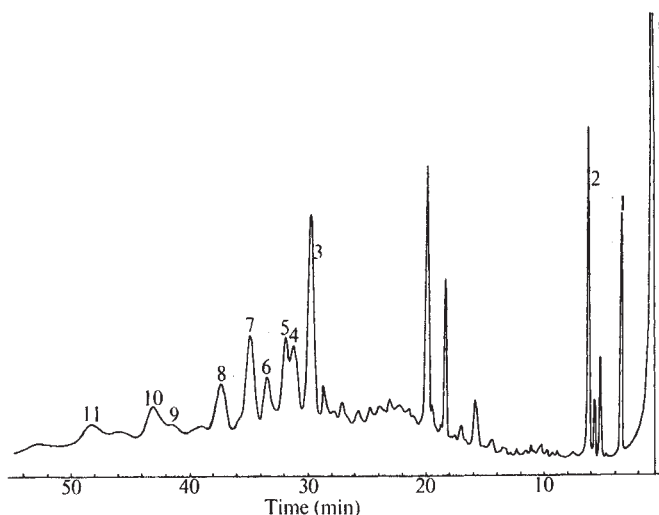
THE chemical composition of succinite (Baltic amber), its botanical origin, and methods of distinguishing it from other fossil resins, are long standing questions<sup>1</sup>, the third of which has been largely solved in recent years by infrared spectrometry<sup>2-4</sup>. In his survey, Langenheim<sup>5</sup> emphasizes the botanical origin and the strong hold which Conwentz's postulated amber source *Pinus succinifera* has had over subsequent workers. This concept has also dominated ideas of the resin's composition; recent suggestions that this largely derives from dimerized abietic acid<sup>6,7</sup> are not persuasive.

About 20% of the resin dissolves in cold ether. Gas-chromatography of this part before and after methylation with diazomethane shows a complex chromatogram (Fig. 1). The main group of peaks is due to methyl esters of abietane and isopimarane acids, and lesser amounts of agathic and dihydro-agathic acids. The identity of these compounds was confirmed by their retention times on three different stationary phases (OV-1, XE-60, and PEGA), and subsequently by combined gas-liquid chromatography and mass spectroscopy. Methyl  $\Delta^8$ -isopimarate (I) was isolated as the crystalline ester (m.p. and mixed m.p. 69.5–71° [ $\alpha$ ]<sub>D</sub><sup>20</sup> +100° in CHCl<sub>3</sub>) (refs. 8 and 9) while methyl sandaracopimarate (II), dehydroabietate (VI), and dihydro- $\Delta^8$ -isopimarate (IV) were isolated in sufficient purity for their identities to be confirmed by i.r.<sup>10</sup>. The hitherto undescribed  $\Delta^8$ -isomer of dimethyl dihydroagathate (VIII) was prepared from the parent ester by acid isomerization. Table 1 shows percentage composition. Eight other samples of Baltic amber also showed the characteristic i.r. spectrum of succinite and gas-chromatograms qualitatively and often quantitatively similar to the isolates. Amounts of the lower MW materials were very variable, however.



We examined the insoluble bulk of succinite by alkaline hydrolysis with results similar to those reported previously<sup>11,12</sup> except that we find that mild conditions are required (N methanolic NaOH at RT for 24 h). The ether-insoluble fraction of the hydrolysed product gives an i.r. spectrum almost superimposable on that of the polymer from kauri resin (from *Agathis australis*). Thomas<sup>13,14</sup> has commented on the similarity of the spectrum of amber to that of kauri and this becomes much closer after hydrolysis. Furthermore the hydrolysed amber polymer and the kauri polymer have similar optical rotations, [ $\alpha$ ]<sub>D</sub><sup>20</sup> +18° and +16° (in pyridine) respectively. Kauri polymer consists of a co-polymer of communical acid (X) and communol (XI)<sup>15,16</sup>, and we propose a similar constitution for the amber polymer though more extensively cross-linked and with the hydroxyl groups partly acylated with succinic acid—long known to be both a free and an esterified component of amber. As expected, succinylation of kauri polymer yielded a product of similar i.r. spectrum to amber.





**Fig. 1** Gas-chromatogram of ether-soluble fraction of succinite after methylation with diazomethane. Column, 9' x 1/4" glass, 1% OV-1 on 80-100 mech diatomite 'CQ', programmed 100-210° at 5° per min. Carrier gas argon at 45 ml. per min. Flame detector. Percentage compositions (in parentheses) were determined from another chromatogram using manool as an internal standard. Peak 1, dimethyl succinate; 2, borneol; 3, methyl  $\Delta^8$ -isopimarate (I, 0.40%); 4, methyl dihydro- $\Delta^8$ -isopimarate (IV, 0.22%); 5, methyl sandaracopimarate (II, 0.20%); 6, methyl isopimarate (III, 0.19%); 7, methyl dehydroabietate (VI, 0.23%); 8, methyl abietate (V, 0.13%); 9, dimethyl dihydroagathate (VII, 0.03%); 10, dimethyl dihydro- $\Delta^8$ -agathate (VIII, 0.05%); 11, dimethyl agathate (IX, 0.06%). Other peaks unidentified.

The results suggest an overall resin composition high in labdane units, with lesser amounts of abietanes and pimarines. This is supported by selenium dehydrogenation results (Table 1) obtained using an empirical correlation between compositions and amounts of typical dehydrogenation products (L. J. G., unpublished work). Succinite yields dehydrogenation products typical of the labdane skeleton<sup>17</sup>.

The diterpenoid composition, however, may not represent that originally present in the resin. The three isopimaric acid isomers readily interconvert (at equilibrium the  $\Delta^8$ -isomer is dominant), and the same is true of the abietadiene acids. We find also that the usual  $\Delta^8$ (17)-labdenes are readily converted to the  $\Delta^8$ -isomers by acids or thermally. Dehydroabietic acid and the dihydro-acids are possibly products of hydrogen exchange.

Comparisons may be made between succinite and modern conifer resins. Many *Pinus* resins, particularly those from subgenus *Diploxylon*, are characterized by a high abietane content<sup>18</sup>. These resins do not seem to polymerize or survive for long periods<sup>19,20</sup>, whereas resins with a high labdatriene content (those from most genera of the *Cupressaceae*, some of the *Taxodiaceae*, and most *Agathis* spp.) start to polymerize as soon as they have issued from the tree and, when they are

produced in sufficient amount (as from *A. australis*), do indeed survive in quantity in the soil. A number of *Pinus* resins, mostly of the subgenus *Haploxylon*, contain large amounts of labdane compounds<sup>21,22</sup>, but more than small amounts of communic acid have not been found. Neither *Larix*, *Cedrus* or *Abies* resins contain significant amounts of labdatriene acids. *Picea* and the remaining genera of the *Pinaceae* can hardly be assessed since few resins have been examined.

Many bark resins of the *Cupressaceae* and *Taxodiaceae* have been surveyed (L. J. G., unpublished work). Although various resins contain several of the compounds detected in succinite none has been shown to contain abietic acid, and in general they do not have a high communol to communic acid ratio. Furthermore, diterpenoid phenols, not detected in amber by gas-liquid chromatography or ultraviolet spectroscopy, are widespread in these families.

Succinite resembles most closely the resins from some *Agathis* species<sup>16,23</sup>, in which isopimaranes, abietanes, and agathic acid are present with much communic acid and communol. Only four species of *Araucaria*, the other genus of the *Araucariaceae*, have so far been examined<sup>24-27</sup> and these show rather varied compositions with marked affinities to *Agathis*. The substantial degree of esterification of succinite, however, is not found in fossil *Agathis* resins of similar age<sup>13</sup>, and in spite of the considerable chemical similarities, the paucity of appropriate botanical remains associated with succinite argues against an *Araucarian* origin.

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**Table 1** Approximate Compositions in Terms of Skeletal Type of Succinite and Some Other Resins Determined by Selenium Dehydrogenation

|                                          | Labdane | Pimarane | Abietane |
|------------------------------------------|---------|----------|----------|
| Succinite (batch analysed)               | 77      | 20       | 2.8      |
| Ether-soluble fraction                   | 58      | 35       | 6.7      |
| Ether-insoluble fraction                 | 83      | 15       | 1.7      |
| Succinite                                | 82      | 17       | 1.5      |
| Succinite                                | 75      | 21       | 4.0      |
| Kauri resin ( <i>Agathis australis</i> ) | 83      | 13       | 4.5      |
| Ether-insoluble polymer                  | 96      | 3.2      | 0.7      |
| Commercial dimerized rosin               | 12      | 22       | 66       |
| * <i>Pinus sylvestris</i> resin          | 0       | 22       | 78       |
| * <i>Pinus cembra</i> resin              | 30      | 35       | 36       |

\* The two *Pinus* resins are examples from the subgenera *Diploxylon* and *Haploxylon*.

# BOOK REVIEWS

## UFOs Analysed

*The UFO Experience: a Scientific Inquiry.* By J. Allen Hynek. Pp. xii + 276 + 10 photographs. (Henry Regnery: Chicago, May 1972.) \$6.95.

MOST sightings of "unidentified" flying objects prove to be apparitions quite familiar to a suitable expert in observational astronomy, meteorological optics or satellite tracking. During the power cuts earlier this year there were reports of numerous strange aerial lights over London: they turned out to be the stars, which are apparently unfamiliar to many city-dwellers. Such reports tend to annoy the scientists condemned to receive them, and it is scarcely surprising that UFOs have acquired such a bad name. Even so, the existence of "genuine" UFOs has not been conclusively disproved, and those who scoff at the subject should ask themselves whether they would believe in meteorites if meteorites were made of solid carbon dioxide and were reported only by naïve observers, who feebly admitted that the objects vanished before scientists could arrive to examine them.

Professor J. Allen Hynek was responsible for setting up the Baker-Nunn satellite-tracking network when he was Assistant Director of the Smithsonian Astrophysical Observatory, and during the 1960s he was astronomical consultant to the USAF project for studying UFO reports. He is therefore very well qualified to write a scientific account of the subject: if you want to make up your mind about UFOs this is the book to read.

From the mass of reports Professor Hynek selects only the small minority he regards as genuinely unidentified. To each he assigns a "strangeness index" depending on the number of items needing to be explained, and a "credibility index" depending on the number and reliability of witnesses (Normally he excludes reports by one witness only.) He divides the reports into six types: nocturnal lights; daylight disks; radar-visual reports; and close encounters of what he calls the first, second and third kind. Encounters of the first kind are those producing no tangible effect;

the second kind covers those said to produce physical effects, such as marks on the ground or interference with car engines; the third kind comprises the "little green men" and their kin.

Professor Hynek is a believer in UFOs, in the sense that he believes there are numerous unexplained and significant reports, which not only deserve to be studied scientifically as "new empirical observations", but should also yield significant conclusions if correctly analysed. A correct analysis is very difficult, because the evidence is mad-deningly unsatisfactory and the analyst would need a strong grasp of diverse physical and behavioural sciences. But Hynek believes the solution to the UFO problem could produce a "mighty and totally unexpected quantum jump" in the development of science.

Will Hynek convince his readers? Every reader approaches this subject trailing clouds of prejudice: all I can do is to declare my own prejudice, and give my own answer. The past experience which has generated my prejudice has been acquired through making 6,000 visual observations of artificial satellites: in the past 15 years I have spent hundreds of hours scrutinizing the night sky with 11×80 binoculars capable of revealing all sunlit objects more than 50 cm in diameter at distances up to 1,000 km. I have not yet seen an unidentifiable object, and I began this book a militant unbeliever. I finished it still unbelieving, but much less militant, for Hynek's catalogue is cumulatively impressive and he convincingly refutes many of the common slanders, such as the idea that most UFO-sighters are psychologically odd. But the best argument in favour of UFOs is still the familiar one, that all striking new advances in science are at first treated with derision by scientists; consequently, some of the phenomena now treated with derision will eventually become respectable, following in the footsteps of evolution, space travel and nuclear power.

The book has its faults, which Hynek should correct in a second edition. For example, he should discard some dubious reports, or show more zeal in trying to resolve them instead of just

saying they are inexplicable. He gives no indication that he has considered the wide variety of meteorological phenomena, such as mock suns and complex halo formations which are fruitful sources of sightings. He also fights shy of the wider issues raised by comparisons with psychic phenomena and cargo cults, and the other semi-religious aspects of UFOs. No doubt he wished to preserve his down-to-earth image; but a full assessment of UFOs must include discussion of their place in the general cultural pattern.

D. G. KING-HELE

## Cajal on the Retina

*The Structure of the Retina.* By S. R. Cajal. Compiled and translated by S. A. Thorpe and M. Glickstein. Pp. xxxix + 196. (Charles C. Thomas: Springfield, Illinois, February 1972.) \$12.50.

THIS is primarily a translation from the German translation by R. Greeff (1894) of Cajal's *La Rétine des Vertébrés* (*La Cellule*, 9, fasc. 1; 1892). The translators incorporate also material from Cajal's final published version (Cajal, S. R. *Lab. Invest. Biol.*, Madrid, 28; 1933).

With the advent of electron microscopy and single unit recording in the central nervous system there has been a notable resurgence of interest in the anatomical data of Cajal and his contemporaries. This has been especially true in recent years for the vertebrate retina. The translation is thus very welcome and will hopefully stop the quotation of Cajal by authors whose text may then reveal that they have not read what he wrote. Wherever I have checked the translation with the original it is well done and follows exactly, as far as I can judge, what the author intended to say. For modern readers unfamiliar with the discussions and controversies of the period this may sometimes make for difficult reading. But the purpose of the translators was to make Cajal's retinal work available in English, not to provide an historical commentary. In an introduction the translators explain clearly how they have translated words like "cones



jumeaux (Zwillingszapfen)" and the sense in which Cajal used "spongio-blast", and so on.

A translation of Greeff's introduction is included. The references are more conveniently placed at the end of the chapters instead of, as in the original, in footnotes. Useful additions are a list of Cajal's papers on the visual system of vertebrates and invertebrates; a name and a subject index, so that one can quickly glean a summary of Cajal's commentaries on various authors, other animals and types of cells. The original plates are very well reproduced considering the difficulties of copying and reducing the size of the figures. Compared with my copy of the 1894 edition there is, however, an occasional slight loss of definition of some of the finer processes of the cells. Ironically this sometimes makes them more closely resemble what may at first be seen in a Golgi preparation.

B. B. BOYCOTT

## Dartians to Martians

*Not from the Apes.* By Björn Kurtén. Pp. viii + 183. (Victor Gollancz: London, June 1972.) £1.75.

BJÖRN KURTÉN already has an established reputation as the author of readable books on vertebrate palaeontology, such as *The Age of the Dinosaurs* and *Pleistocene Mammals of Europe*. In *Not from the Apes*, he has turned his hand to the vexed question of human evolution. He attempts to explain to the layman a subject which is still the centre of animated discussion among the experts, who are generally reluctant to write such books at this stage of research. This is why *African Genesis*,

which was written by a former playwright (Robert Ardrey), was eagerly read by many who were impatient to read any kind of summary of the discussion of human origins. There is no justification, however, for "popularizing" material not yet established as scientifically sound. It is therefore unfortunate that Kurtén should have based his book on the poorly supported hypothesis that the ancestors of man and the apes separated more than 35 million years ago. This flaw greatly reduces the value of an otherwise enjoyable book which quite neatly summarizes human evolution from the Australopithecines ("Dartians") onwards. The reader is largely presented with opinions, not facts, and this cannot be justified merely as a means of avoiding complexity.

It rarely emerges from the text that the author's opinions are based on a handful of fossil fragments of *Propliopithecus* and *Ramapithecus* which are supposed to represent 30–35 million years of human evolution before *Australopithecus*. A sense of proportion could have been kept by illustrating all of these fragments on one page. Such is the "historical evidence" of which Kurtén states: "I am afraid that we cannot dispense with key characteristics; fortunately the teeth give us all the necessary ones as far as apes and man are concerned." Kurtén does admit, however, that the scientist "cannot reconstruct a hitherto unknown animal on the basis of a single tooth or even the whole tooth row. . . . This is the situation with *Ramapithecus*." One of the few illustrations in this book shows a "reconstruction" of the entire skull of *Ramapithecus*!

Kurtén is, of course, aware that the

African great apes and man show remarkable similarities in chromosome and protein structure; but he concludes that these are due to parallel evolution. He does not consider the possibility that some of the morphological similarities between *Propliopithecus*, *Ramapithecus*, *Australopithecus* and *Homo* might be parallel developments. Further, Kurtén overlooks the fact that it is very unlikely that similar amino-acid sequences will arise independently in two animal species, and that such changes are accessible to mathematical treatment. The possibilities of parallel evolution in mammalian dental and cranial anatomy can be estimated only crudely and subjectively. Some serologists—such as Sarich—have admittedly taken the opposite view that the fossil record can be largely ignored when constructing evolutionary trees, and have concluded that the ancestors of African apes and man separated only five million years ago. This view is just as reprehensible as the claim that the fossils can tell us all we need to know; neither approach is desirable.

Let us hope that the readers of this book, on seeing the section suggesting that man might develop as a new species on Mars, will realize that Kurtén should not be taken seriously.

R. D. MARTEN

## Addendum

IN Dr Cornwall's review of the *Atlas of Animal Bones for Prehistorians, Archeologists and Quaternary Geologists* (Bilingual Bones, *Nature*, 238, 474; 1972), the name of the author, Elizabeth Schmid, was unfortunately omitted. We apologize for this oversight.

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